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# Spatial & Temporal Patterns of Microplastic Pollution in Wellington, New Zealand, and the Southern Ocean

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## Abstract

The global marine environment is currently facing unprecedented anthropomorphic change and stress. One such stressor is plastic pollution, which has continually increased in magnitude since mass production began in the 1940's. An increase in plastic debris throughout the oceans not only results in an infiltration of the pollutants throughout the entirety of the marine environment, but also increases the risk that it impacts the physiological, structural, and behavioural traits of various organisms – including humans. These negative interactions are particularly likely with microplastic particles (< 5 mm), as they can enter and be transferred throughout the food web with ease. However, research in the field of microplastic pollution is extremely one-sided, with most present studies focusing on the Northern Hemisphere. Additionally, comparatively little has been investigated regarding temporal and spatial patterns of microplastic occurrence. The aim of this research was to 1) examine the abundance and distribution of synthetic particles in sub-surface waters of the Southern Ocean, across broad temporal and spatial scales and 2) examine finer-scale spatial and temporal patterns of microplastic load within the urbanised Wellington Harbour, New Zealand, using a combination of environmental and biological indicators.

To assess the broad-scales of temporal and spatial variation in the Southern Ocean, annual Continuous Plankton Recorder (CPR) tows were undertaken between New Zealand waters and the Ross Sea, Antarctica, over a span of 9 years (the austral summers of 2009/10 – 2017/18) and a range of 5 oceanographic zones and two frontal systems, totalling a distance of approximately 22,000 km. Overall, patterns were inconsistent, with no constant increase or decrease in load throughout the years, while spatial variation was minimal and not associated with particular oceanographic fronts or proximity to an urban area. Despite no consistent spatial variation, temporal differences did occur between years. Again, there were no identifiably consistent trends across years (i.e. a gradual increase), but there was a substantial peak in 2009/10 and a trough in 2012/13. Such changes are likely due to large-scale variations in ocean circulation systems, along with environmental drivers such as El Niño and La Niña events.

To investigate the microplastic load in a more urbanised environment, 3-monthly surveys were undertaken with surface waters, beach sediments, and *M. gallloprovincialis* mussels in Wellington Harbour, New Zealand, using samples from three sites for beach and mussel

surveys, and two sites for the surface water tows. Weekly variation was also measured for beach sediments and mussel tissues. Again, no consistency was observed in temporal or spatial variation for any environmental or biological indicator, however the average pollutant loads were on par with reported results in other literature, particularly for *M.*

*galloprovincialis* tissues. Temporally, the peak microplastic load in the tissues of the mussel, *M. galloprovincialis*, appeared to correlate with the peak load found within the surface waters of the harbour, indicating a possible relationship between plastic pollution in the environment and that which is found within organisms. Finally, the spatial variation observed within beach sediments was far larger than that seen throughout the mussel tissues, supporting the idea that beach sediments are microplastic sinks, but also susceptible to a range of environmental drivers including wind strength, wind direction, and sediment erosion.

Throughout the Southern Ocean and within Wellington Harbour, particle characteristics were similar, in that microfibrils were the prevailing synthetic morphotype – accounting for upwards of 90% of all particles found. These results are similar to reports from other current literature, but not associated with public knowledge that is currently in the media and represented in the legislation. The results of this thesis illustrate the importance of monitoring and managing the occurrence and effect of microplastics on both fine- and broad-scales of temporal and spatial variation and helps address the knowledge gap surrounding microplastics in the Southern Hemisphere.

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## 1. General Introduction

Ensuring the health and productivity of the world's oceans is integral to maintaining global biodiversity and the earth's continued ability to thrive. However, numerous stresses are impacting the marine environment and its ability to support biodiversity on a global scale, including climate change, ocean acidification, and pollution. One key source of pollution is plastic debris, which has recently been described as any object that contains a synthetic or semi-synthetic polymer as the essential component, solid in natural environments and insoluble at 20°C (Hartmann et al., 2019). However, in general, plastic as a material can be defined as any synthetic organic polymer which is derived from oil and gas monomer polymerisation (Derraik, 2002; Rios et al., 2007; Thompson et al., 2009; Cole et al., 2011). As a result, plastics are extremely diverse in size, colour, chemical composition, and origin; they exist throughout the world as a key material that is used daily.

The commercial manufacture of plastic took off in the 1940's, around the time of the Second World War (Cole et al., 2011). Since this first instance of mass production, the development and use of plastic items has continued to grow, with the material's seemingly inexhaustible purposes cementing its place in modern society. This rise in popularity can be attributed to its key characteristics, strength, durability, and flexibility. The latest data shows that 359 million tonnes of plastic were produced globally in 2018 alone (Figure 1.1), the highest amount produced in a single year since commercial manufacture began (PlasticsEurope, 2019). However, this figure is likely to be highly conservative, as it does not account for synthetic fibre production or direct microplastic production (Hermabessiere et al., 2017).

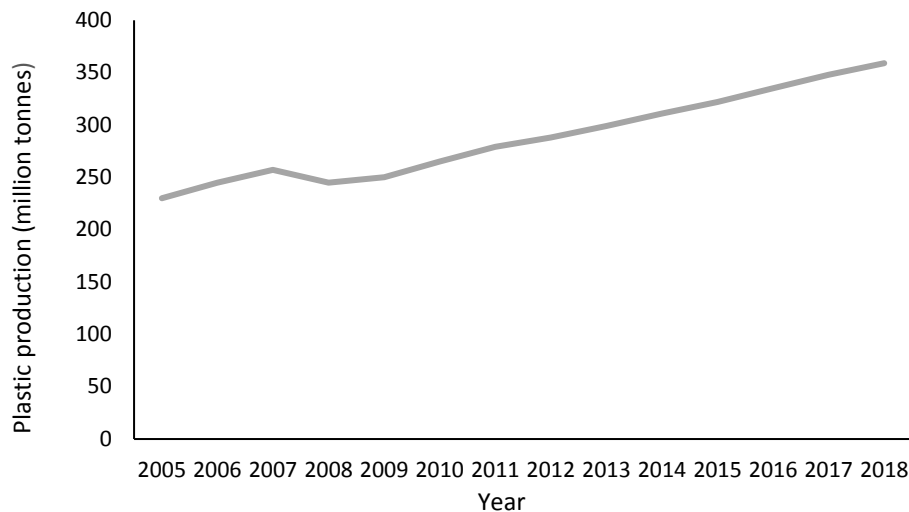


Figure 1.1: Data from PlasticsEurope (2019) market data, demonstrating the global growth in plastic production from 2005 - 2018, measured in million tonnes.

### *The problem with plastic*

Considering the sheer amount of annual plastic production that has been occurring for decades, and the diverse range of uses the material has throughout human society, it is unsurprising that plastic debris also exists in many forms. In fact, the characteristics of plastics that make them invaluable materials within human society are precisely the features that enable them to persist in the natural environment as long-lasting pollutants (Derraik, 2002). Such debris can be found along an extensive size array, ranging from large commercial fishing nets that have been discarded, through to microscopic beads from beauty products (Fendall & Sewell, 2009; Napper *et al.*, 2015). Consequently, plastic is ubiquitous around the globe and spread across terrestrial, freshwater, and marine biomes. Plastics are associated with numerous interactions and negative impacts on a wide range of ecosystems. For example, microplastics (<5 mm) within terrestrial environments are thought to leach chemicals into the surrounding soil and environment, leading to negative effects upon organisms such as plants and earthworms (Chae & An, 2018). However, despite plastic being manufactured by humans on land, a vast majority ends up in the marine ecosystem (Derraik, 2002; Thompson *et al.*, 2004; Eriksen *et al.*, 2014; Galgani *et al.*, 2015). Plastic litter makes its way into the ocean via several different pathways, including direct inputs such as littering, and run-off of manufactured consumer products (Reisser *et al.*, 2013). Conversely, indirect inputs such as chemical and physical weathering of larger plastic debris inevitably results in fragmentation of the synthetic polymers into smaller particles that are continuously decreasing in size (Andrady, 2011; Jambeck *et al.*, 2015). This inability to successfully

biodegrade causes a high abundance of plastic debris to infiltrate the world's oceans – from the sea surface to the benthos of the deep sea, and polar regions to the tropics (for example: Goldberg, 1997; Obbard *et al.*, 2014; Woodall *et al.*, 2014; Van Sebille *et al.*, 2015). The numerous pathways to the marine environment are exacerbated by contributing factors such as low regional and national plastic recycling rates. The recycling of these materials is often limited, due to the wide variety of plastic polymers available for use, which complicates the ability of governments to develop adequate methods for recycling, in ways that will not be detrimental to the environment (Dauvergne, 2018).

To compound this issue, once in the marine environment, many plastic polymers are initially less dense than the surrounding seawater, so can be transported over long distances via surface currents (Maes *et al.*, 2017). However, as factors like weathering and biofouling alter the appearance and structure of plastics, the buoyancy of the material decreases, thus promoting mixing of the pollutants throughout other parts of the water column (Ye & Andrady, 1991; Morishige *et al.*, 2007; Maes *et al.*, 2017). As a result, these synthetic materials interfere with many different marine species and taxa through direct contact such as entanglement and ingestion. Given that plastic comes in many different sizes and shapes, it is bioavailable to many different marine organisms. Size ranges of plastic includes nanoparticles (1-100 nm), microplastics (1-5000µm), and macroplastics (>5 mm) (Hartmann *et al.*, 2019). It can therefore enter the food web at various levels, from primary consumers to apex predators (Derraik, 2002; Van Freneker *et al.*, 2011).

However, it is not only the direct interactions that organisms have with marine debris that presents a problem. In contrast to the initial school of thought – that plastic litter is a long-lasting yet inert pollutant - the production of plastic materials involves the addition of chemicals such as plasticizers and flame retardants which enhance the durability and functionality of the material (Andrady, 2017). Consequently, as fragmentation occurs in the ocean, these chemicals leach into the surrounding environment where they become available for ingestion by marine biota (Oehlmann *et al.*, 2009). Leaching is not the only toxicity issue that arises from plastic pollutants, as Persistent, Bioaccumulative, and Toxic substances (PBT's) known as Persistent Organic Pollutants (POPs) are efficiently bound to synthetic polymers (Andrady, 2011; Engler, 2012). Although POPs are naturally found in seawater at low concentrations, they can accumulate at a faster rate on the surface of plastic debris due to both compounds being hydrophobic (Engler, 2012). Consequently, the ingestion of plastic debris not only establishes the presence of synthetic particles within the food web, but also

introduces POPs to marine biota at unnatural levels (Bakir *et al.*, 2012; Andrady, 2017). Chemical interactions such as these can impact the health and mortality of organisms (Rochman *et al.*, 2016; Andrady, 2017), but are also deleterious at non-lethal levels, through the alteration of behaviours like predation efficiency (De Sá *et al.*, 2015).

### *Abundance & Distribution of plastic litter*

Plastic pollution is rightly considered a global environmental issue, as there is a high abundance of both macro- and microplastic pollution along a wide spatial gradient. Plastic has been observed and documented in most marine environments from the poles to the equator, in urbanised and remote oceanographic regions. The local, national, and international extent of this pollutant impacts the effect that it may have on marine biota. For this reason, a comprehensive portrayal of the global plastic pollutant load and distribution within the oceans, along with how its physical, chemical, and structural characteristics are integral to understanding the risks for various ecosystems. Additionally, the continued increase of global plastic production rates (Figure 1.1), along with its long-lasting nature, complex recycling governance, and the unsatisfactory waste management indicates that plastic is likely to persist, if not increase in prevalence, throughout the marine environment for the foreseeable future (Barnes *et al.*, 2009; Tetu *et al.*, 2019). Consequently, data provided on plastic abundance and distribution may be valuable in focussing minimisation and management efforts.

Estimates vary on how much plastic debris can be found in the world's oceans, as the load can be highly variable due to factors such as widely ranging oceanographic conditions, seasonal effects, and spatial differences in plastic inputs. Despite this complexity, Cole *et al.* (2011) suggests that marine plastic debris likely accounts for 10% of all plastic produced. Conversely, Hermabessiere *et al.* (2017) stated that a low estimate for global, floating plastic litter may be between 70,000 – 270,000 tonnes. Combinations of intensive sea-surface sampling and statistical modelling indicates that microplastics comprise 1% of the plastic debris that enters the oceans each year from land-based sources (Van Sebille *et al.*, 2015). However, one type of microplastic, microfibers, are thought to account for up to 95% of the sea surface, benthic, and coastline litter (Cesa *et al.*, 2017; Galgani *et al.*, 2015). Three comprehensive studies have been undertaken with the aim of determining microplastic occurrence, utilising various methodologies and oceanographic models. Cózar *et al.* (2014) estimated between 7000 – 35,000 tons of plastic (size range 0.20 – 100 mm), while Eriksen *et al.* (2014) suggested 35,500 metric tonnes (size range 0.33 – 200 mm). Van Sebille *et al.*



(2015) produced a considerably higher estimate than previous studies of 93,000 – 236,000 metric tonnes (size range <200 mm). Despite these estimates being highly variable and including meso-particles larger than 5 mm, cumulatively they highlight the sheer magnitude of the issue.

Abundance estimates are likely conservative and do not show the full extent of the problem. Currently, sea surface tows and oceanographic models are the primary measures available to monitor microplastic. Eriksen *et al.* (2014) recognises this limitation, particularly after considering how macroplastic fragmentation impacts the prevalence of microplastics. Theoretically, since large debris degrades into smaller particles, the volume of microplastics in the marine environment should be comparable to the volume of macroplastic. In addition, these global estimates only account for microplastics on the sea surface, and does not include those suspended in the water column, on the benthos, within sediment and sea ice, or ingested by organisms, which together likely comprise a large fraction of what is in the oceans (Eriksen *et al.*, 2014). Sampling efforts may also impact the abundance of particles, with a wide range of collection methods and weather events disrupting sampling. Strong winds, for example, decrease surface microplastic concentrations by as much as five times (Collignon *et al.*, 2012; Kukulka *et al.*, 2012; Ivar de Sul & Costa, 2014). Additionally, other removal processes impact the abundance and distribution of surface microplastics, including decreased particle buoyancy (Barnes *et al.*, 2009) and biodegradation of some synthetic polymers by microbes (Shah *et al.*, 2008).

Along with aiding fragmentation, physical processes help to determine where plastic litter is distributed. Although plastic debris has been documented in all major oceans, factors such as ocean circulation and wind-driven vertical mixing increase the likelihood of its concentration in certain oceanographic regions (Van Sebille *et al.*, 2015). Consequently, plastics at the sea surface are of highest densities in the convergence zones of sub-tropical gyres (Cózar *et al.*, 2014; Kanhai *et al.*, 2017). Cózar *et al.* (2014) describes this convergence as a large conveyor belt that collects floating plastic debris from the continents and brings it together using large-scale vortices. Van Sebille *et al.* (2015) identifies the North Pacific Gyre as containing the largest global reservoir of plastic litter. This is the result of its proximity to plastic inputs by the populous United States and Asia, along with the convergence of Ekman transports (Jambeck *et al.*, 2015; Van Sebille *et al.*, 2015). Eriksen *et al.* (2014) estimates that 55.6% of the total floating plastics are in the Northern Hemisphere, 37.9% of which are confined within the North Pacific.

Despite occurring in high concentrations at convergence zones, plastic debris also infiltrates many other habitats and ecosystems, including both the deep sea and polar regions – For example within sea ice. Although often far-removed from urbanisation and human society, habitats such as the deep sea and its associated benthos have been reported to contain a microplastic load of up to one particle per 25 cm<sup>2</sup> in the Mediterranean and Atlantic Ocean (Van Cauwenberghe *et al.*, 2013). Synthetic fibres were also documented in similar regions, with concentrations reaching up to four orders of magnitude higher than microplastics surveyed at the sea surface (Woodall *et al.*, 2014). Larger plastic debris has also been documented within the deep-sea benthos of the Arctic (Bergmann & Klages, 2012). As the deep-sea is considered a sink for plastic litter (Goldberg, 1997), it can be inferred that both micro- and macroplastics are likely to be a typical part of the sediment layer throughout all major ocean basins.

Sea ice, on the other hand, typically incorporates microplastics as opposed to larger plastic debris. Formation of ice concentrates whatever particles are encompassed within it, trapping it until the ice melts (Obbard *et al.*, 2014). This was the first publication that illustrated direct evidence for substantial microplastic pollution in the Arctic (Lusher, 2015). Obbard *et al.* (2014) sampled ice cores from remote locations around the North Pole, with the subsequent results showing concentrations two-times higher than the surface waters throughout the Atlantic or the North Pacific gyre at the same time (38 – 234 particles per m<sup>3</sup>). As sea ice is a temporary sink for microplastics, it is highly likely that amplified annual sea-ice melt due to ever-increasing effects of climate change, will result in the release of a large amount of microplastics into the surrounding water column where they are able to negatively interact with organisms (Obbard *et al.*, 2014; Peeken *et al.*, 2018).

## *Macroplastics*

### *Research trends – Spatial & temporal patterns*

Throughout the second half of the 20<sup>th</sup> century, the rise in global plastic production coincided with the understanding that plastic did, in fact, pose a problem to the oceanic environment. Consequently, scientific studies began to investigate the impacts of the pollutant, primarily focussing upon macroplastic debris as it can be detected within the environment more easily, as well as detecting deleterious effects upon organisms from interactions such as entanglement and ingestion. Moreover, publications that demonstrated the direct impact of plastic on marine biota played a meaningful role in altering society's view of the pollutant,

aiding a perspective shift from plastic debris being an inert and insignificant pollutant, to one that can interfere with physiology, behaviour, and mortality of organisms.

The quantification of macroplastic abundance and distribution has long been a secondary focus to the direct effects that these materials have upon organisms. In fact, a recent paper by Ostle *et al.* (2019) was one of the first that investigated long-term trends in surface water macroplastic abundance. This study demonstrated a clear increase in plastic litter over the 60-year time series (1957-2016), across the North Atlantic. Although this is the most extensive study on how marine plastic debris has changed over time, previous studies have also indicated similar trends. For example, a report by Ryan & Moloney (1993) demonstrates an exponential increase in litter on an uninhabited island in the Tristan da Cunha group of the South Atlantic Ocean, between 1984-1990. Interestingly, both studies also note how, despite the clear increase in debris within the marine environment, research and monitoring surrounding the issue dropped off after 1987 (Ryan & Moloney, 1993; Ostle *et al.*, 2019). A visual database of more than 1,000 marine litter studies – LITTERBASE (Tekman *et al.*, 2019) – illustrates that the number of macroplastic and microplastic abundance and distribution studies are similar (Bergmann *et al.*, 2017; Ostle *et al.*, 2019), but also most widely distributed around heavily populated regions (Figure 1.2). Considering that microplastic pollution is an emerging field, it is surprising that there are also significant gaps in the temporal and spatial trends regarding macroplastic pollution. In general, there appears to be a consensus that more work needs to be undertaken to understand patterns surrounding plastic pollution for all size classes, although visual databases such as LITTERBASE are a useful first step to recognise areas that require further attention (Bergmann *et al.*, 2017; Ostle *et al.*, 2019).

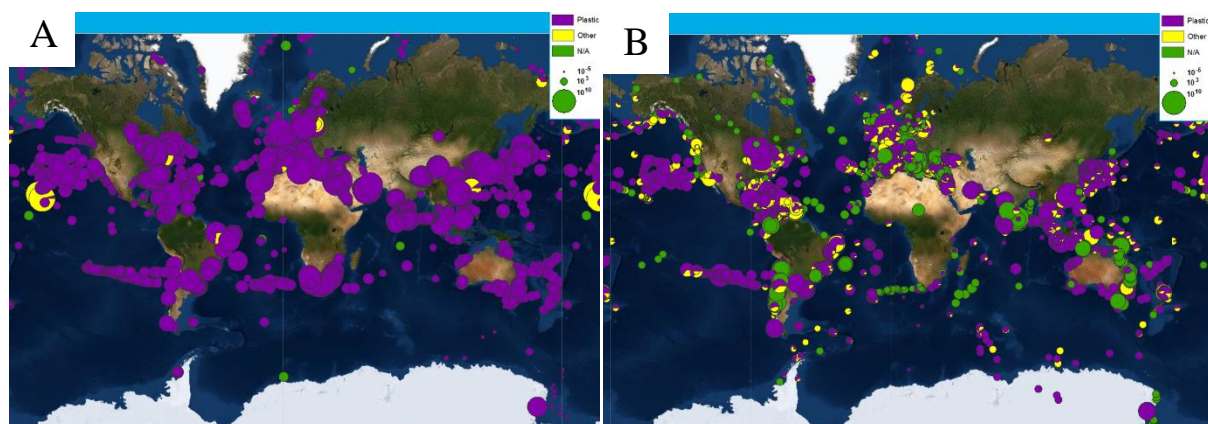


Figure 1.2: Distribution and results of studies investigating A) macroplastic abundance, and B) microplastic abundance across the globe (Tekman *et al.*, 2019), where size of circle indicates relative reported pollutant concentrations.

### *Research trends – Interactions with biota*

Although there are distinct research gaps regarding long term distribution modelling and monitoring of macroplastic, the impacts that this pollutant has had upon marine organisms has been well described in the literature. Most of this literature centres around ingestion and entanglement, including many of the early studies and reports. In 1947, for example, a herring gull was reported to be entangled in a string (Jacobson, 1947; Ostle *et al.*, 2019). Ten years later, a piece of twine – assumedly from fishing trawls – and a plastic bag were found tangled in a Continuous Plankton Recorder (CPR) (Ostle *et al.*, 2019). At this stage, only a decade or so had passed from the beginning of commercial plastic production, yet there was already evidence of this pollutant being found in the open ocean.

Entanglement in plastic debris became a persistent problem for marine organisms almost from the time that this synthetic material entered the ocean. The issue has been well-understood for decades (Law, 2017), but the threats has only become more prevalent as the amount of plastic litter in the oceans increases. Evidence now suggests that approximately 46% of seabird species, 100% of sea turtle species, and 26% of marine mammal species are negatively impacted by plastic entanglement, often resulting in serious injury or death (Koelmans *et al.*, 2014; Worm *et al.*, 2017). Although entanglement directly impacts individuals, this threat has been linked to the population decline of different species, including seals and sea lions (Fowler, 1987; Henderson, 2001; Derraik, 2002). One common cause of entanglement is discarded fishing nets (Figure 1.3), often referred to ghost fishing nets (Worm *et al.*, 2017). For marine air-breathing taxa such as turtles, seabirds, and mammals, the inability to breathe due to entrapment in a net will likely have severe consequences. There is a myriad of other plastic items that are able to wrap around, choke, and encase organisms, with the immense variety of plastic shapes and sizes exacerbating the number of species and taxa that are vulnerable to plastic entanglement (Worm *et al.*, 2017). In addition to impaired breathing, the other key consequences for individuals affected by entanglement include suffering abrasions and deep cuts into tissue from a tightly-wound item, as well as a negative effect upon mobility; thus, becoming vulnerable to predation (Derraik, 2002). Additionally, problems may arise when juveniles become tangled in plastic pollution. As the individuals grow, they are likely to become constricted and impaired by the material. Entanglement is an issue typically constrained to macroplastic pollution, as microplastics are generally not large enough to entrap an organism. Consequently, past research has heavily focussed upon the effects of entanglement, as the results are both easily noticeable and

severe. Entanglement presents a significant problem to countless marine species, particularly as it is not only thought to impact individuals, but rather the fitness and success of populations.

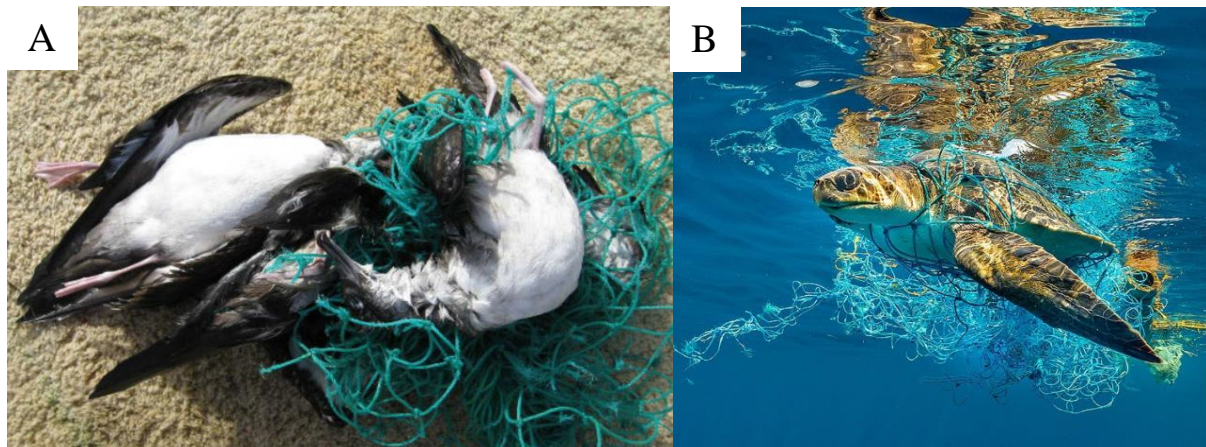


Figure 1.3: Images of A) a dead shearwater seabird and B) a sea turtle, both entangled in a common form of marine plastic pollution; discarded fishing nets. Image credit: A) NOAA Fisheries Pacific Islands Regional Office, B) Brian Skerry, National Geographic photographer.

Within addition to entanglement, ingestion of macroplastic is also extremely common, with countless anecdotal and scientific reports of marine animals being found with some form of plastic in their digestive system (Derraik, 2002). This was first illustrated in the 1960's with seabirds (Figure 1.4) and marine turtles (Harper & Fowler, 1987; Gregory, 2009; Law, 2017), however, like the issue of entanglement, research is still being undertaken to demonstrate the exact impacts of macroplastic ingestion and how widespread the problem is across taxa and oceanographic location. Ingestion may either be passive, such as the act of filter-feeding, or active, which may involve mistaking the item for prey (Derraik, 2002). Active ingestion is a mechanism that is common amongst marine biota such as sea turtles, who regularly mistake items such as plastic bags for a primary prey source, jellyfish (Figure 1.4) (Mattlin & Cawthorn, 1986; Derraik, 2002; Worm *et al.*, 2017). This interaction is not only cited often in scientific literature, but also widespread in the general media and often credited as the inspiration behind plastic bag bans in numerous nations (Xanthos & Walker, 2017; Avery-Gomm *et al.*, 2018). Compared to microplastic ingestion, the larger and easier-to-observe size of macroplastic often results in its direct interactions with biota being more commonly reported in the media and in peer-reviewed publications, especially since this type of marine debris has extensive consequences for charismatic megafauna like sea turtles, seabirds, and cetaceans. The size of the debris also means that fewer items are required to cause gastrointestinal blockages, with gradual starvation a readily cited negative impact of plastic ingestion, in conjunction with chemical leaching (Derraik, 2002).



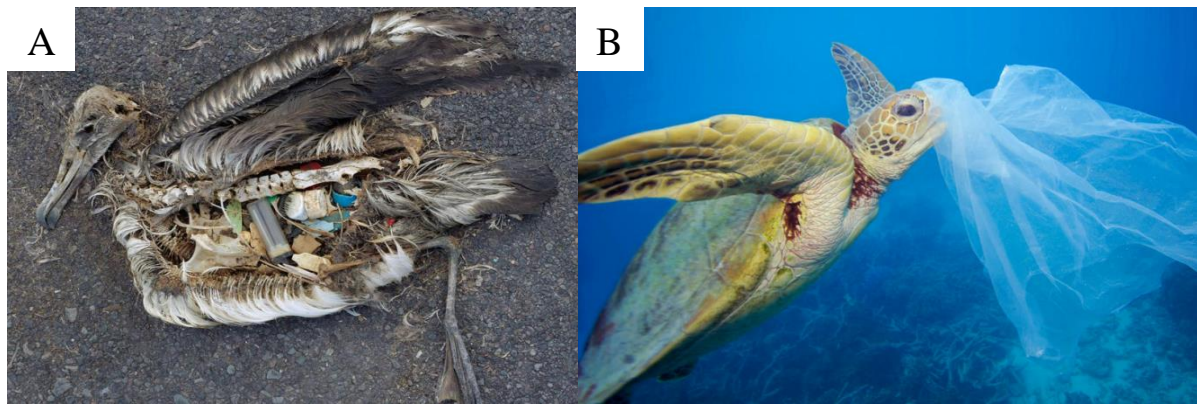


Figure 1.4: Images of A) a deceased Laysan Albatross with ingested plastic debris in its internal tissues and B) a sea turtle in the process of eating a discarded plastic bag. Image credit: A) Chris Jordon, Smithsonian Museum, B) Troy Mayne, WWF.

## Microplastics

### *Origin & history of microplastic research*

In contrast to the substantial research of macroplastics and their effects on marine biota, microplastics are a relatively new and emerging research topic within the field of marine pollution. Although there has been an increasing focus on these particles within the scientific community since the early 2000's (Ivar do Sul & Costa, 2014), much is still unknown regarding their abundance, distribution, and impacts on organisms in the marine environment.

The first publication solely focused on plastic  $< 5$  mm in length/diameter was released in 1971 and was the only report or paper to discuss the issue of synthetic fibres in the marine environment before the rise of the 21<sup>st</sup> century (Ivar do Sul & Costa, 2014). Even though this was the first paper of its kind in the scientific literature, Buchanan (1971) recognised that the particles appeared to be steadily increasing in abundance. Furthermore, despite the field of microplastic research having only recently emerged, with fewer than 20 papers published pre-2000, the research commonly recognised the potential consequences that these synthetic particles could have on the marine environment and the function of its ecosystems. Carpenter and Smith (1972) discussed how increasing concentrations of such particles may lead to a high accumulation on the sea surface. Similarly, Colton et al. (1974) emphasised the inability of plastic debris to biodegrade, which is an issue then confounded by the particles' ability to provide an ideal surface for PCB's to grow. However, most of the early papers generally observed and documented the abundance and distribution of microplastics in Northern Hemisphere regions such as the Sargasso Sea (Carpenter & Smith, 1972), the North-western Atlantic (Colton et al., 1974), coastal New England (Carpenter *et al.*, 1972), and the Bristol Channel (Morris & Hamilton, 1974).

Although early papers had a primary focus on determining where microplastics were found, as well as how abundant they were, interactions with and effects on marine biota were also often recognised. This included the ingestion of particles by seabirds (Hays & Cormons, 1974; Fry *et al.*, 1987; Ryan., 1988; Van Franeker & Bell, 1988) and fish (Carpenter *et al.*, 1972; Kartar *et al.*, 1973), although the effects on these organisms remained unclear, due to the biological implications being more difficult to show or test for (Colton *et al.*, 1974). However, it was assumed by early researchers that microplastics were unlikely to be beneficial to any animal (Gregory, 1996; Zitko & Hanlon, 1991). Early publications also began to speculate on the sources of such pollution, and how efforts could be made to minimise the introduction of further litter. Zitko & Hanlon (1991) demonstrated, for example, that plastic scrubbers from facial cleansers could be attributed to some of the direct microplastic inputs into the marine environment in the forms of polyethylene, polypropylene, or polystyrene. In addition, they proposed that from a ‘common sense basis’, use of such products – as well as unnecessary discharge – should be minimised, otherwise accumulation will likely occur in the marine environment.

### *Interactions with biota*

Due to their small size, microplastics are often biologically available for marine organisms. Consequently, both direct and indirect interactions such as ingestion and subsequent trophic transfer are a widespread issue in many different ecosystems. A plethora of studies have examined ingestion of these synthetic particles, along with the issues microplastics may cause for various individuals, populations, and species. Unlike the ingestion of macro debris, microplastics often enters the food web at the lower trophic levels, for example marine invertebrates (Lusher, 2015). However, it has also been shown that marine alga can absorb microplastic debris by incorporating the particles into their cellulose, resulting in measurable oxidative stress and decreased photosynthetic output (Battacharya *et al.*, 2010). However, whether ingestion occurs as the result of accidental consumption or selective choice often depends on the relative concentration of plastic associated with normal prey items, as well as the organism’s ability to discriminate between prey and plastic litter (Moore *et al.*, 2001; Moore, 2008).

Although many macroplastic ingestion studies focus on larger marine organisms, most microplastic ingestion studies are carried out at lower trophic levels, particularly with smaller benthic and planktonic invertebrates. These organisms are commonly filter-, suspension-, or deposit-feeders, and are therefore highly susceptible to microplastic ingestion from the water

column and sediments. However, whilst it is straightforward to demonstrate that an animal has ingested or absorbed plastic debris, it is more difficult to determine how long plastic debris remains in an organism's digestive tract and tissues, as well as any physiological, structural, or behavioural effects. A few such studies have reported evidence of decreased feeding rates in zooplankton (Cole et al., 2013) and lugworms (Besseling et al., 2013), while increased mortality was observed in copepods after generational exposure to the toxicity of microplastics (Lee et al., 2013). In addition to feeding and survival, fitness of marine invertebrates is also thought to be adversely affected. Oysters experience reduced fecundity and decreased offspring quality, both of which are attributed to the ingestion of microplastic particles (Galloway & Lewis, 2016; Sussarellu et al., 2016).

### *Conclusion and Aims of research*

Plastics are an important pollutant in the oceanic environment, regardless of size, shape, or type. They are now recognised as being long-lasting and ubiquitous in abundance and distribution worldwide. Furthermore, both microplastics and macroplastics have significant and adverse effects upon marine organisms; ranging from primary producers through to apex predators. These effects may be direct, such as entanglement or ingestion of plastic debris. Alternatively, indirect effects are also experienced, including the ingestion of leached chemicals from the surface of plastic, or the trophic transfer of synthetic materials that may lead to bioaccumulation of both physical debris and/or chemical toxicity.

Efforts are continuously being made to expand the understanding of how plastic pollution impacts marine organisms, but it is also vital to understand where the debris is found and in what quantities. Such information enables research on biological effects to focus upon organisms particularly susceptible to direct impacts, as well as oceanographic regions with high pollutant loads. Most of the studies within this review have been carried out in the Northern Hemisphere, leaving parts of the Southern Hemisphere greatly under-researched and lacking reliable plastic pollution metrics (Avio *et al.*, 2017). In particular, studies undertaken to assess plastic load within the remote oceanographic region of the Southern Ocean have only recently started to be carried out, and New Zealand's microplastic research has only recently had a resurgence (e.g. Clunies-Ross *et al.*, 2016; Webb *et al.*, 2019; Bridson *et al.*, 2020) after a strong beginning in the 1970's (Gregory, 1977; Gregory, 1978). Increasing awareness surrounding microplastic patterns throughout the world's oceans is also integral, as the fragmentation of larger plastics already in the marine environment will likely result in an increasing amount of smaller synthetic particles in the coming decades.



Consequently, this thesis explores the abundance and distribution of microplastics over temporal and spatial scales, to identify patterns in both a remote area of the ocean, as well as a heavily urbanised setting. The first chapter examines surface water microplastic load over a 10-year time series in the Ross Sea Region of the Southern Ocean. The second chapter surveys the inner harbour of Wellington, New Zealand, encompassing surface water, sediment, and biological samples. to gain a broad perspective of microplastic pollution across multiple surfaces. Up-to-date assessments and information on microplastic load and its distribution should aid in developing plans and legislation to manage and mitigate the spread of global oceanic plastic pollution.

## 2. Large-scale temporal and spatial patterns of microplastic pollution between New Zealand and the Ross Sea, Antarctica

### *Abstract*

This study aimed to characterize the pollutant load of synthetic particles in a multi-year, large scale study between New Zealand waters and the Ross Sea Region of Antarctica.

Field collections were made during each austral summer (November – March), between the years 2009/10 – 2017/18. Sub-surface tows collected plankton and inorganic material such as microplastic with a 270 µm mesh silk, using a Continuous Plankton Recorder (CPR).

Microplastic abundance, colours, sizes, and morphotypes were counted and subsequently quantified over temporal and spatial scales.

There was no evidence for consistent patterns of change across the sampled months, years, or latitudinal zones, however pollutant loads were often dynamic in their occurrence. Yearly abundances varied substantially, with a large peak in the 2009/10 sampling year, and a deep trough in 2012/13. These responses suggest that floating microplastic debris is likely at the mercy of ocean circulation systems and large-scale environmental drivers such as El Niño and La Niña effects. Regarding particle characteristics throughout the sub-surface waters of the Southern Ocean, a broad range of both colours and sizes were found. In total, 9 different colours were identified across the samples, and these ranged in size from < 1 – 5 mm.

Overwhelmingly, however, the most significant result regarding particle characteristics came from the morphotypes that were observed. Microfibres accounted for 90.6% - 99.5% of all particles collected each year, which supports the figures reported in other literature.

## Introduction

Antarctica and the surrounding Southern Ocean is considered, on a whole, to be the most remote geographical region in the world, with the relatively recent increase of scientists and tourism operations becoming the only regular visitors to the icy continent (Hughes, K.A., 2010; Shah, R.M., 2013). However, the ubiquity of plastic pollution would suggest that debris is likely to have also permeated these waters. This is especially probable when considering the oceanography of the Southern Ocean and the current system that drives it. In comparison to the remote nature of the Antarctic land mass, the Southern Ocean is integrally connected to the rest of the world. The Antarctic Circumpolar Current (ACC) wraps around the Antarctic continent and thus is connected to the Atlantic, Indian, and Pacific oceans (Figure 2.1) (Rintoul, *et al.*, 2001; Rintoul, 2018). This oceanic circulation system, the ACC, both thermally isolates the Southern Ocean to prevent excessive melting of ice, and transports nutrient-rich, warmer water that has originated from low latitudes around the continent (Tynan, 1998; Martinson, 2012). This large-scale, circumpolar circulation provides a pathway for pollutants such as microplastics to be introduced to and established within the waters of the Southern Ocean.

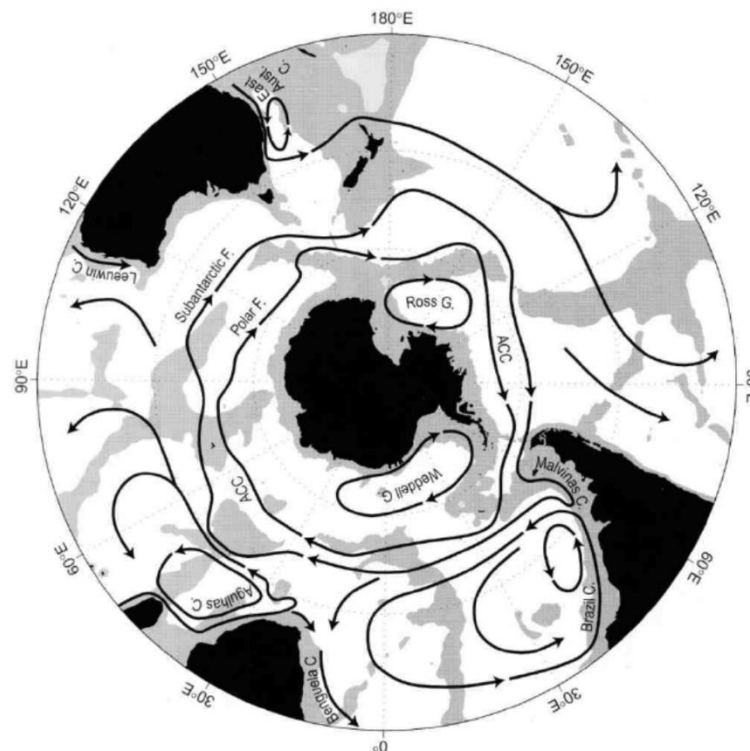


Figure 2.1: Schematic depiction of major currents of the Southern Ocean, with particular reference to the Antarctic Circumpolar Current (ACC) and its associated fronts, which encircles the Antarctic continent. Image credit: Rintoul *et al.*, 2001.

Research into plastic pollution throughout the polar regions has been a recent development, with many studies focussing on the more accessible and urbanised parts of the ocean. Before 2014, no studies had shown any direct evidence for a substantial microplastic pollutant load in the Arctic (Lusher, 2015), and it wasn't until 2017 that researchers began sampling sediments and surface waters around the coast of Antarctica (Cincinelli *et al.*, 2017; Isobe *et al.*, 2017; Munari *et al.*, 2017; Waller *et al.*, 2017). Prior to this, only two scientific papers had been published on plastic debris in the Southern Ocean, one on macroplastic occurrence (Barnes *et al.*, 2010) and the other on ingestion by petrels (Van Franeker & Bell, 1988). More recently, abundance and distribution estimates around the Antarctic continent have been carried out by multiple research teams from numerous nationalities, with an emphasis on using data from the surface waters of the Southern Ocean to explain how macro and microplastic pollution loads are impacted by oceanic currents and their rate of dispersal around the globe (e.g. Isobe *et al.*, 2019). Plastic pollution in polar regions is still an emerging research topic, and recent papers examining the abundance of microplastics in Antarctic waters are highly variable. In general, publications accept that microplastics are found within multiple environments in the Southern Ocean, including sediments and surface waters. In Admiralty Bay, microfibers had an average abundance of  $2.40 (\pm 4.57)$  particles per  $100 \text{ m}^{-3}$  across 60 samples in the 2010-11 austral summer (Absher *et al.*, 2019). Similarly, a study carried out around the Antarctic Peninsula found an average abundance of meso- and microplastics of  $0.008$  particles per  $\text{m}^{-3}$  (Lacerda *et al.*, 2019). Altogether, Southern Ocean microplastic abundances have been poorly classified, with most publications focused on a localised area such as the Antarctic Peninsula or a specific bay (Absher *et al.*, 2019; Lacerda *et al.*, 2019). Such a lack of broad spatial and temporal studies is largely due to the logistical difficulties of carrying out research in this isolated region of the world.

The Southern Ocean, covering the region south of  $60^\circ\text{C}$  (Stössel *et al.*, 2015), covers a vast expanse of ocean, so to limit microplastic studies to a narrow temporal or spatial range is to limit the knowledge that can be obtained on how extensive this pollutant is. Between New Zealand and the Ross Sea Region lies five major oceanographic zones and three main frontal systems (Figure 2.2), where each frontal system and its associated latitudinal zones have distinct characteristics and biological associations (Robinson *et al.*, 2014; Rintoul, 2018). For example, satellite observations have recorded distinct differences in sea surface temperature and sea surface height across the different zones (Moore *et al.*, 1999; Solokov & Rintoul, 2009; Graham *et al.*, 2012) The Sub-Tropical Front (STF) and its associated Sub-Tropical

Zone (STZ) have been suggested to be more susceptible to wind shifts, as this region is surface intensified (Graham *et al.*, 2012). However, the other frontal systems, located at higher latitudes and within the ACC, are reported to be more sensitive to the associated topography, as opposed to wind shifts (Graham *et al.*, 2012). Differences between the frontal systems are also observed when regarding biological communities. For example, Tynan (1998) and Rodhouse & White (1995) reported that the ecological community found within the Polar Frontal Zone (PFZ) includes cephalopods, but krill and fishes were absent. The spatial variability between frontal systems not only helps to control the global climate (Graham *et al.*, 2012), but is also possibly a factor that contributes to the distribution of microplastics throughout the Southern Ocean, along with the interactions that these synthetic particles may have with the ecological communities which inhabit these waters.

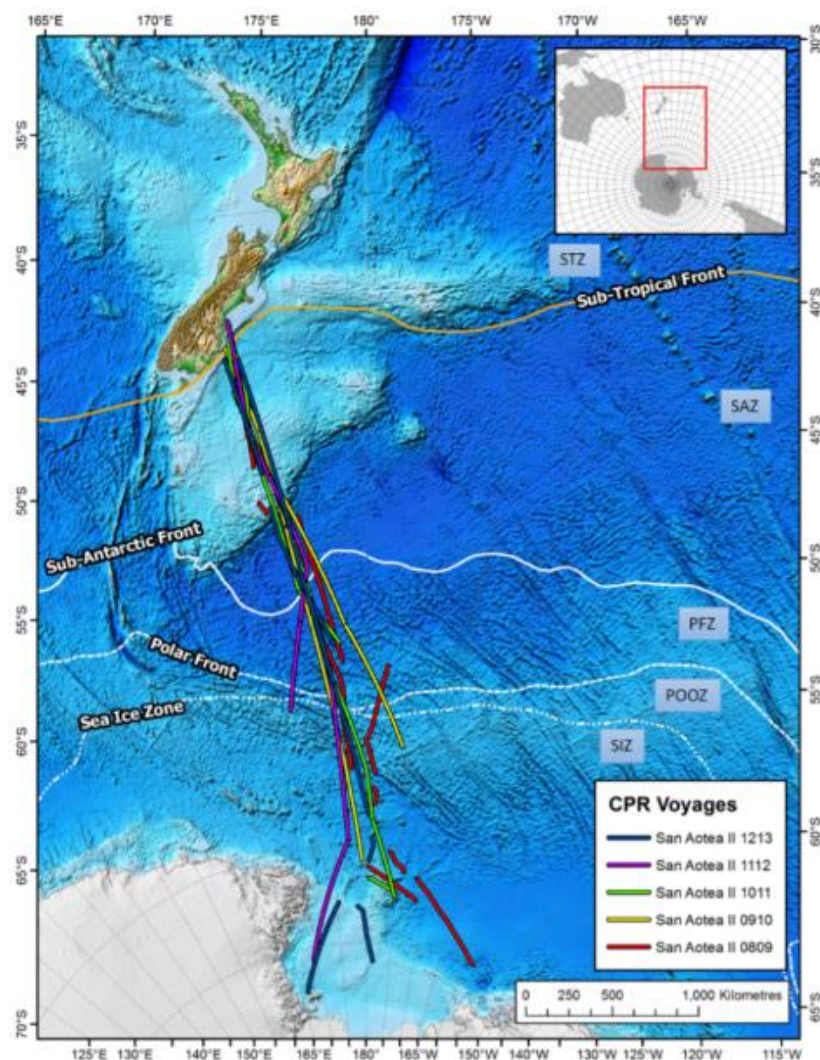


Figure 2.2: CPR tows undertaken by FV *San Aotea II* during the first five annual sampling voyages (2008/09 – 2012/13), where transects can be seen traversing the five latitudinal zones and three frontal systems.

Another contributing factor to the abundance and distribution of floating marine microplastic pollution is time. With the ever-increasing amount of plastic that is produced – and consequently discarded into the environment each year – temporal variation is an integral aspect of microplastic research. Currently, the only multi-year, long-term temporal study investigating floating debris has been carried in the Northern Hemisphere (Ostle *et al.*, 2019). This study took place over a period of 60 years (1957-2016) and recorded a clear increase in macro- and microplastic pollution throughout sub-surface waters in the North Atlantic. On the other hand, Isobe *et al.* (2019) conducted a survey in the Southern Hemisphere, between Japan and Antarctica, in the Austral summer of 2016-2017. This study, while not a long-term temporal field study, utilised a numerical model to predict likely microplastic abundances in the future – up to 2066. This study by Isobe *et al.* (2019) also predicted that there will be an unmistakable increase in microplastic loads throughout these southern waters in the next ~40 years. This limited understanding of long-term temporal patterns surrounding microplastic research severely limits the future advancement of this field, as mitigation efforts depend largely upon a well-defined knowledge of its distribution and abundance. Without a solid grasp of these long-term patterns, models will not be able to effectively predict the dispersal or occurrence patterns of this pollutant throughout the world's oceans.

The aim of this chapter is to characterise the trends in abundance as well as size and colour of microplastics in sub-surface tows over a 9-year sample period. I examine the abundance of synthetic particles and their spatial distribution over a ~22,000 km (11,900 nautical mile) transect between New Zealand and the Ross Sea, within and between years. I hypothesized that the prevalence of microplastics will increase with the proximity to urbanised, New Zealand waters, but that there will be significant variation in microplastic abundance between years, due to random dispersal effects and environmental variables. In the same way that other scientific literature has found fibres to be the most predominant type of microplastic within surface waters, I also hypothesized that these types of particles will have the highest abundance within the study area and time period.

## Methods

### Data collection

Field sampling occurred every austral summer from 2008, between New Zealand and the Ross Sea Region (defined here as 160°E – 150°W and South of 40°S), as seen in Figure 2.3. The collection of sub-surface water samples was directed by NIWA (National Institute of Water and Atmospheric research), in collaboration with the Antarctic toothfish fishing vessel, FV *San Aotea II*. Detailed descriptions of field methodology, along with the initial post-voyage sampling, can be found within the Robinson *et al.* (2014) Ministry for Primary Industries (MPI) report. Briefly, samples were collected by the FV *San Aotea II* as it followed a similar route to and from its Southern fishing grounds each year and also had long periods of continued cruising/steaming, both factors allowing for adequate sampling durations and annual repetitions (Figure 2.3). Water samples were collected while in transit between New Zealand and the Ross Sea Region, using a Continuous Plankton Recorder (CPR).

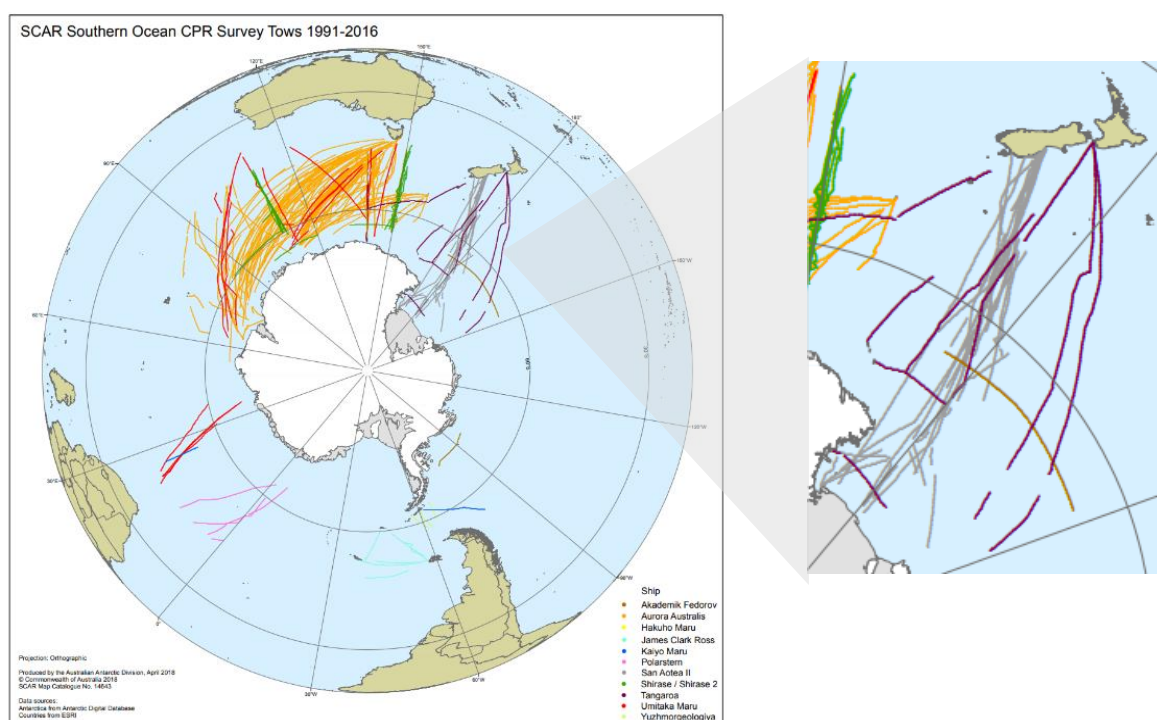


Figure 2.3: A depiction of SCAR Southern Ocean CPR survey tows between 1991-2013, with particular focus upon the light grey transects conducted by the FV *San Aotea II* for this study, in conjunction with NIWA. Image credit: Australian Antarctic Data Centre © Commonwealth of Australia.

Continuous Plankton Recorders (CPR) were initially created to aid plankton surveys in sub-surface oceanic waters. However, because of their ability to capture small organisms like plankton, they could also be used for the purpose of assessing microplastic load. During this



study, the CPR was towed roughly 100 m astern of the FV *San Aotea II*, at an approximate depth of 10 m below the sea surface. According to the methods described in Robinson *et al.* (2014), the CPR conducted tows whilst the vessel travelled at cruising speed. Throughout each deployment, water, organisms, and inorganic material entered the body of the CPR via a small opening (12.7 x 12.7 mm), before they were passed through a 270  $\mu$ m collecting silk (Figure 2.4). This silk layer was then coated with a covering silk, which consequentially captured animals and inorganic materials between the two layers. During this process, an external propeller moved the silk through the internal workings of the CPR at an approximate rate of 1 cm per 1 nautical mile (nm), regardless of the cruising speed at which the FV *San Aotea II* was travelling. Both silks were finally rolled into a preservation tank that contained a solution of buffered 40% formaldehyde at the rear of the CPR body (Figure 2.4), where they remained until the completion of the tow. Each CPR deployment was able to run for up to a maximum 450 nautical miles (nmi), however the actual tow length was heavily dependent upon environmental factors at play such as wave height and wind strength.

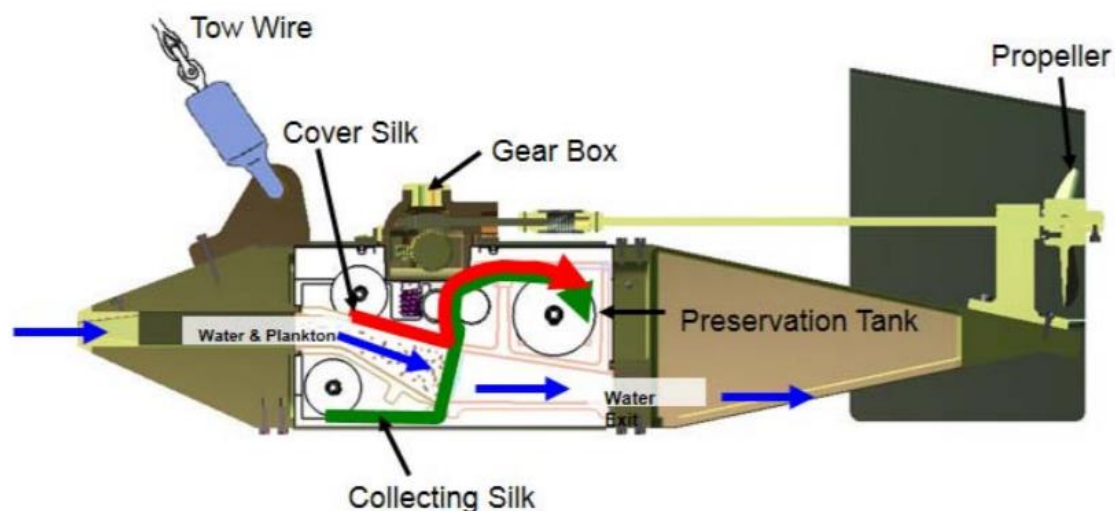


Figure 2.4: A depiction of the internal workings of a Continuous Plankton Recorder (CPR), as used in this study. Image credit: Robinson et al., 2014.

Over the 20 separate transits that were conducted over the 10-year sample period (2008/09 – 2017/18), the CPR was deployed 71 times over a collective distance of 23,286 nmi (43,140.5 km), with an average of 7 tows per transit (Table A2.1). FV *San Aotea II* crew members were trained by NIWA technicians to operate the CPR machinery and the associated chemicals,



tools, and safety equipment effectively. They were also trained in correct preservation methodologies for all samples, so to minimise contamination (Robinson *et al.*, 2014).

### *Laboratory protocol*

#### *Post voyage sampling*

At the completion of each voyage, samples were transported to the NIWA laboratories, where they were cut into 5 cm segments that each represented 5 nautical miles of towed distance (Figure 2.5). These segments were individually analysed to determine Phytoplankton Colour Index (PCI), zooplankton abundance, and taxonomic identification to the highest possible level. Whilst examining samples for the presence and identification of biota, presence and absence of inorganic materials was recorded. All 5 cm segments were preserved in 10% formaldehyde. For tows between 2009/10 – 2017/18, all samples that contained any inorganic material were set aside, and later sent to me to further characterize the microplastics at the Victoria University Coastal Ecology Laboratory (VUCEL). The samples from 2008/09 were not analysed as a part of this study, due to time constraints.

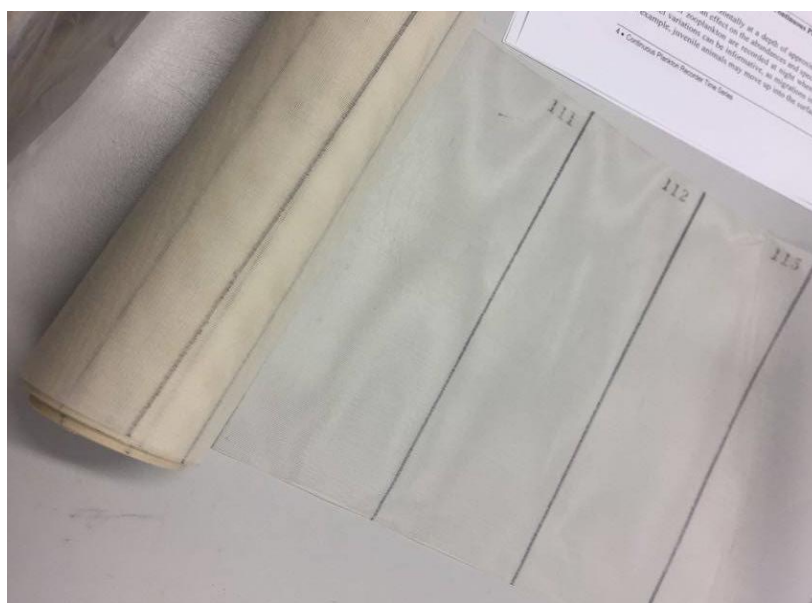


Figure 2.5: A roll of 270  $\mu$ m collecting mesh, which is used in CPR tows throughout this study. Once in the NIWA laboratory, these rolls are cut into the 5 cm samples along the marked lines, at which point they are ready for microscopic analysis.

#### *Sample preparation*

All 1558 samples with at least one piece of inorganic material (37% of the total samples collected between 2009/10 – 2017/18) were individually washed with distilled water, visually analysed, then preserved using an adapted NIWA protocol. To prepare the individual samples for analysis, the two layers of silk were removed, then the 40% formaldehyde preserving

solution was poured through a 75  $\mu\text{m}$  filter. The collecting and covering silks were also rinsed with distilled water and washed through a 75  $\mu\text{m}$  filter, to recover any residual trapped biological or inorganic material.

### *Visual analysis*

Washed samples were transferred to a petri dish (Figure 2.6) and analysed under a stereo microscope at 15x magnification. Synthetic materials were recorded by colour, size, and type. Particles were categorized into three size classes; small (<1 mm), medium (>1 - 2 mm), or large (>2 - 5 mm), determined by measuring each inorganic particle at its longest or widest point. The type of synthetic particle was either recorded as a fibre, fragment, foil, paint chip, or macroplastic (> 5 mm). Finally, the colour of each synthetic particle was also recorded. To verify that the visual identification of a microplastic was correct, forceps were used to attempt to break apart the particle. If it could not be broken, it was determined to be a form of microplastic debris, whilst if it could be broken but was an irregular shape and/or colour, it was classed as a paint chip.

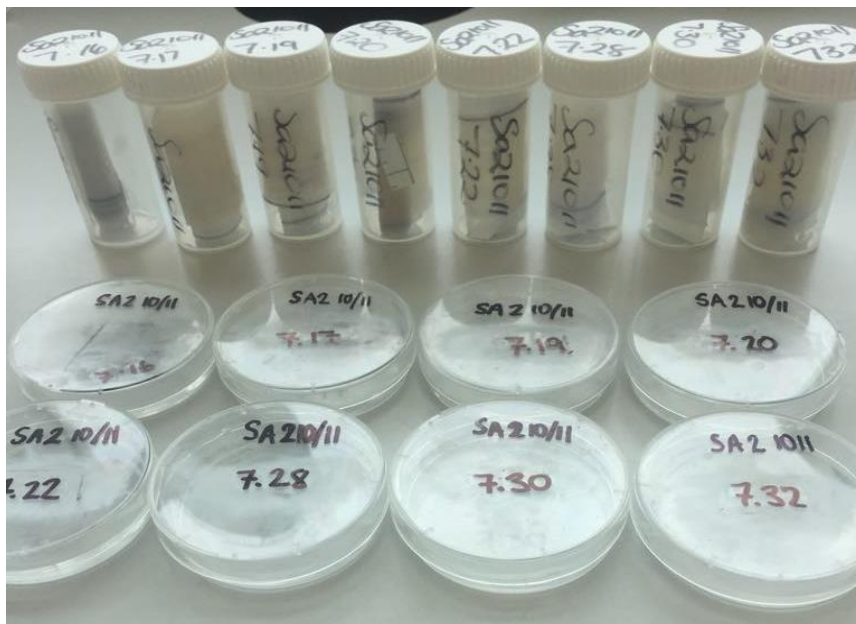


Figure 2.6: Samples arrived at Victoria University Coastal Ecology Laboratory (VUCCEL) in tubes. To prepare each sample for microscopic analysis, silks were removed, and the formaldehyde solution was washed through a 75  $\mu\text{m}$  filter, along with both mesh layers. Samples were then transferred to the pictured petri dishes and labelled appropriately.

### *Statistical analyses*

All analyses were conducted in R V3.6.3 (R Core Team 2020) using RStudio v1.2.5033 (RStudio Team, 2020). Firstly, a Chi-square Likelihood Ratio Test (LRT) was used to

determine whether there was a significant effect of month, transit (North- or South-bound), year, or oceanographic zone. Further analyses were conducted if results were significant. For significant variables, a Poisson mixed effects model was used, with an adjustment made for autocorrelation using the AR1 structure. Pairwise comparisons using the EMMEANS package conducted tests for years that had significantly different counts from each other. Similar tests were conducted for the effects of month and oceanographic zone. Results were given on a log scale.

Pearson's Chi-square tests were conducted for the three aspects of particle characteristics: morphotype, size, and colour. The results of these determined if there was a significant difference between variables.

## Results

Throughout the 9-year sampling period, 4211 individual surface water samples were collected during CPR tows between New Zealand's waters and the Ross Sea Region of Antarctica. Of these, Figure 2.7 demonstrates that there was a high yearly variation in the proportion of individual 5 cm silk samples that contained at least one microplastic particle. The most recent survey, in the austral summer of 2017/18, had a total of 487 samples with 88.3% of these containing at least one synthetic particle (Table 2.1). In contrast, the austral summer of 2011/12 had a total of 404 samples, but only 26.8% of these contained any microplastics (Table 2.1). Overall, the percentage of samples containing microplastic was less than 50% in most years.

Table 2.1: Total number of samples collected from surface waters between New Zealand and the Ross Sea, broken down into the total number of samples containing at least one microplastic particle and those without any microplastics.

Year	Total	Number of samples with $\geq 1$ microplastic	Number of samples with no microplastic particles
2009/10	468	149	319
2010/11	541	192	349
2011/12	404	31	373
2012/13	504	135	369
2013/14	449	187	262
2014/15	454	103	351
2015/16	465	70	395
2016/17	439	260	179
2017/18	487	430	57

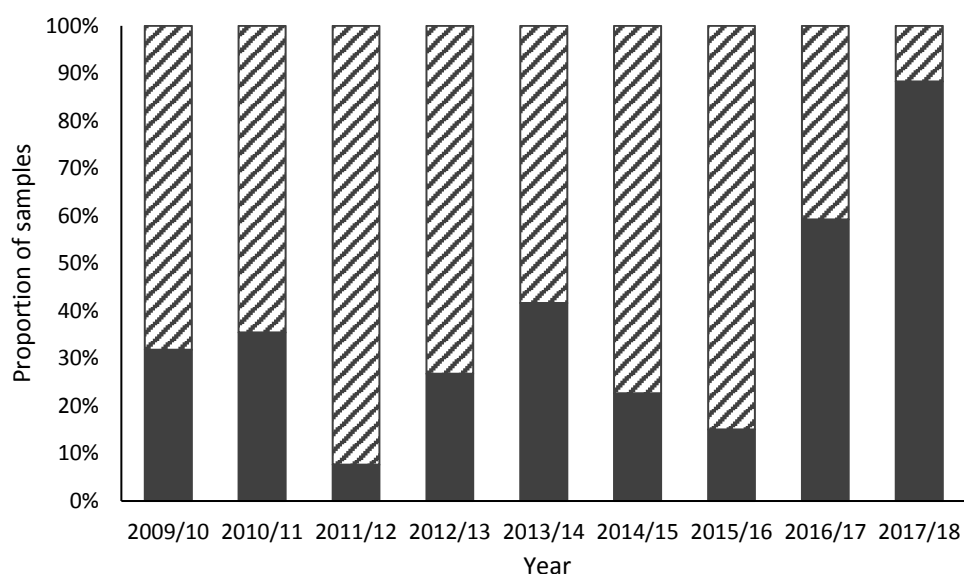


Figure 2.7: Proportion of total samples collected per year, where black bars represent the percentage of samples that contain at least 1 microplastic particle and striped bars represent the percentage of samples that do not contain any microplastics.

### *Explanatory Variables*

For all explanatory variables of microplastic abundance in surface waters between New Zealand and the Ross Sea Region, three were not independent. Both temporal variables (month of collection and year) were significant predictors (Table 2.2) and so was the spatial variable; oceanographic zone. There was no effect of direction of travel, North- or South-bound along the transect, on microplastic abundance (Table 2.2). Since month, year, and oceanographic zone of surface water sampling all have a relationship with mean microplastic abundance, these were able to be investigated further.

Table 2.2: Results of Chi-squared Likelihood Ratio Test examining effects of collection month, collection transit, year, and oceanographic zone on microplastic abundance between New Zealand and the Ross Sea Region. \* indicates significance at  $p = 0.05$ .

	AIC	LRT	P. value	
Month	13126	23.107	< 0.0001	*
Transit	13109	0.05	0.8233	
Year	13134	39.253	< 0.0001	*
Zone	13186	84.332	< 0.0001	*

## *Temporal variation*

### **Between-year**

Average microplastic abundance in surface waters between New Zealand and the Ross Sea Region was variable across years. There was no consistent increase or decrease in average load throughout the 9-year study (Figure 2.8), however the average amount of microplastics found in the 2009/10 survey was significantly higher than 2011/12, 2012/13, and 2013/14 (Table 2.3). In contrast, as shown in Figure 2.8, the 2012/13 samples only contained up to half the amount of microplastics on average compared with 2009/10, 2014/15, and 2015/16 (Table 2.3).

Table 2.3: Results of a pairwise comparison (adjusted for the effect of autocorrelation) examining the effect of sampling year (2009/10 – 2017/18), on microplastic abundance within surface waters between New Zealand and the Ross Sea Region. Results are averaged over the levels of Month, Transit, and Zone, and are significantly different at  $p = 0.05$

Contrast	Estimate	SE	df	t.ratio	P.value	
2009/10 – 2011/12	0.7369	0.182	49	4.042	0.0054	*
2009/10 – 2012/13	0.8728	0.137	49	6.392	<0.0001	*
2009/10 – 2013/14	0.5008	0.120	49	4.191	0.0034	*
2012/13 – 2014/15	-0.4932	0.148	49	-3.327	0.0405	*
2012/13 – 2015/16	-0.7428	0.174	49	-4.269	0.0027	*

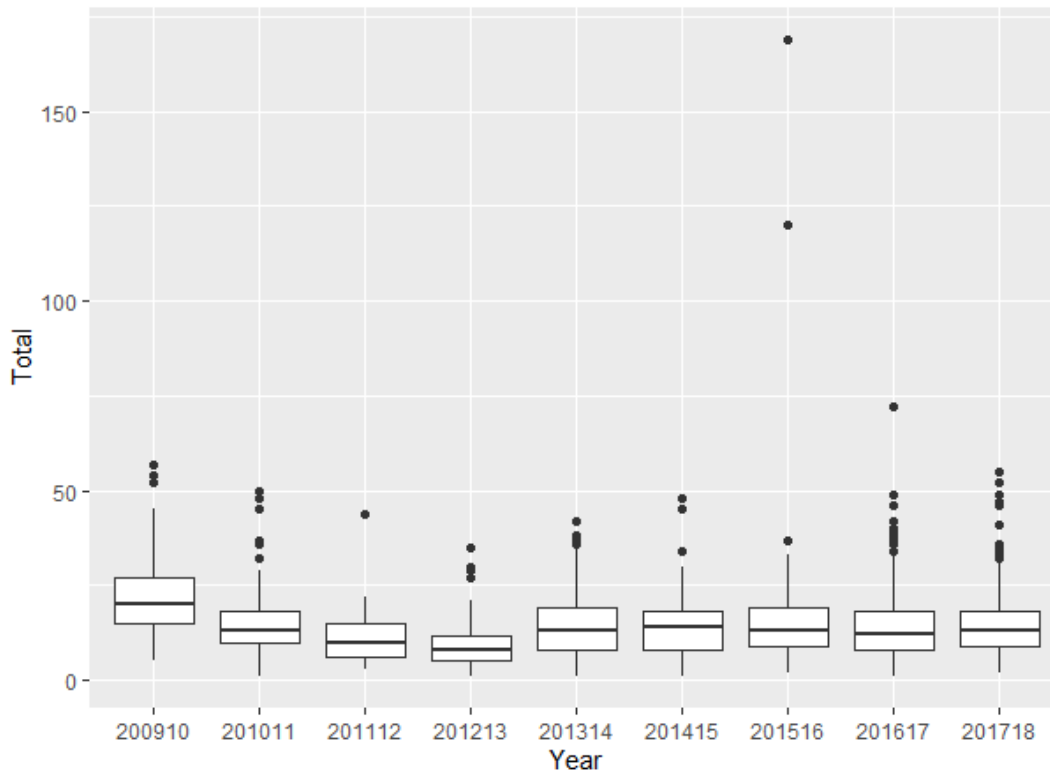


Figure 2.8: Distribution of synthetic particle abundances in surface waters between New Zealand and the Ross Sea, over a 9-year period (2009/10 – 2017/18). Data is pooled across months, transit, and zones.

#### Between-month

Throughout the 9-year annual sampling period, tows were carried out between November and March (austral summer). Similar to the yearly variation, microplastic abundance was also dynamic between months, with no consistent increase or decrease in load throughout the summer (Figure 2.9). The largest difference occurred between the month of December and the following two months, with December having the highest average number of microplastics. However, there was no significant difference between the microplastic abundance in any month where sampling took place (Table 2.4).

Table 2.4: Results of a pairwise comparison (adjusted for the effect of autocorrelation) examining the effect of month (November, December, January, February, March), on microplastic abundance within surface waters between New Zealand and the Ross Sea Region.

Contrast	Estimate	SE	df	t. ratio	P.value
Nov - Dec	-0.0026	0.162	1490	-0.016	1.0000
Nov - Jan	0.3867	0.221	1490	1.750	0.4039
Nov - Feb	0.3195	0.254	1490	1.257	0.7178
Nov - Mar	0.0276	0.399	1490	0.069	1.0000
Dec - Jan	0.3893	0.221	1490	1.766	0.3942
Dec - Feb	0.3221	0.215	1490	1.502	0.5616
Dec - Mar	0.0302	0.374	1490	0.081	1.0000
Jan - Feb	-0.0672	0.129	1490	-0.522	0.9852
Jan - Mar	-0.3592	0.339	1490	-1.060	0.8269
Feb - Mar	-0.2920	0.310	1490	-0.943	0.8800

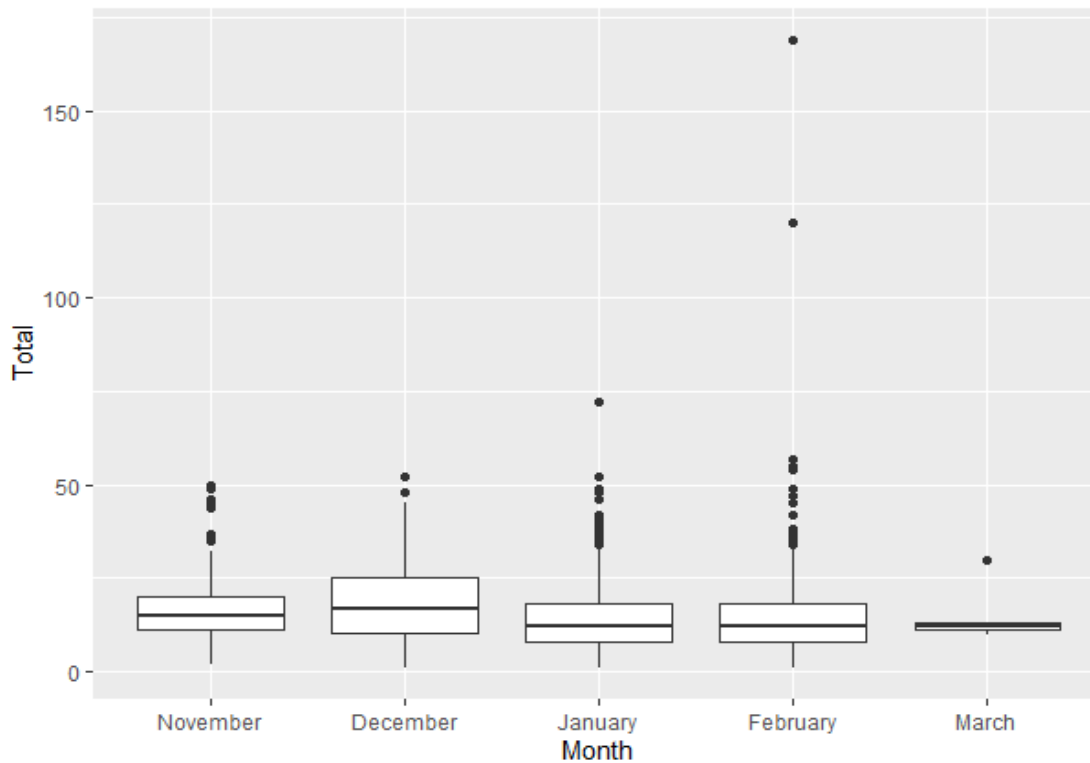


Figure 2.9: Distribution of synthetic particle abundances across the 5 different collection months in the austral summer (November – March), with data pooled across years, zones, and transits.



## Spatial variation

### Overall variation

Figure 2.10 demonstrates the variability that was observed between microplastics throughout all zones and years. There was no discernible pattern or trend observed throughout the 9-year period, or the five oceanographic zones and frontal system where microplastics were collected.

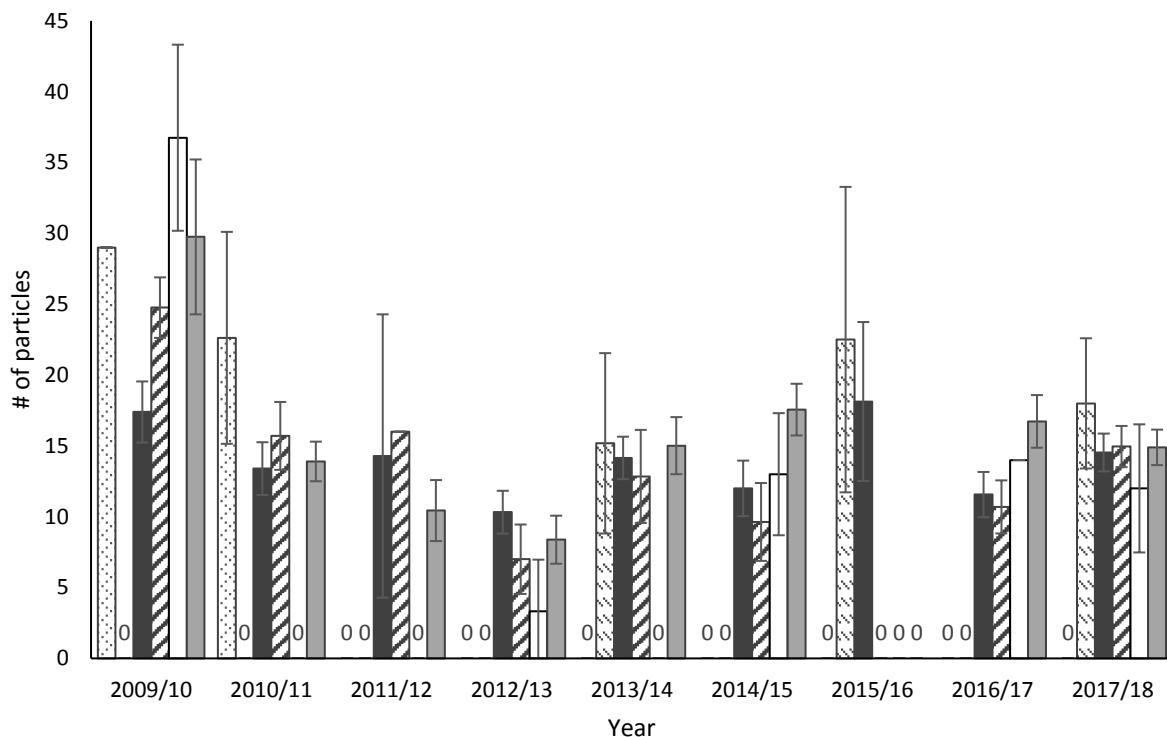


Figure 2.10: Mean ( $\pm$  95% confidence interval) surface water microplastic abundance from the 2009/10 sampling period to 2017/18, between five oceanographic zones and two frontal systems. In order from closest proximity to New Zealand, dotted bars represent the Sub-Tropical Zone (STZ), dashed bars represent the Sub-Tropical Front (STF), black bars represent the Sub-Antarctic Zone (SAZ), striped bars show the Polar Frontal Zone (PFZ), open bars represent the Permanent Open Ocean Zone (POOZ), and grey bars represent the Sea Ice Zone (SIZ). On the graph, 0 demonstrates zones that collections did not occur in.

### Between oceanographic zones

Between New Zealand and the Ross Sea Region of Antarctica, a variety of oceanographic zones and frontal systems were sampled during the annual CPR collections between 2009 – 2019. With data pooled across years, Figure 2.11 demonstrates that there was no noticeable difference in the amount of microplastics collected in most of the different oceanographic zones and frontal systems sampled during this study. However, Table 2.5 indicates that significant differences were observed between the Sea Ice Zone and both the Polar-Frontal (PFZ) and Sub-Antarctic Zone (SAZ).

Table 2.5: Results of a pairwise comparison (adjusted for the effect of autocorrelation) examining the effect of oceanographic zone (PFZ, POOZ, SAZ, SIZ, STF, and STZ), on microplastic abundance within surface waters between New Zealand and the Ross Sea Region. \* indicates significance at  $p = 0.05$ .

Contrast	Estimate	SE	df	t.ratio	p.value	
PFZ - POOZ	-0.14866	0.147	1490	-1.027	0.9090	
PFZ - SAZ	-0.03257	0.0737	1490	-0.442	0.9979	
PFZ - SIZ	-0.24607	0.0759	1490	-3.241	0.0154	*
PFZ - STF	-0.24798	0.1870	1490	-1.326	0.7707	
PFZ - STZ	-0.54669	0.2131	1490	-2.565	0.1065	
POOZ - SAZ	0.11608	0.1512	1490	0.768	0.9728	
POOZ - SIZ	-0.09742	0.1489	1490	-0.654	0.9867	
POOZ - STF	-0.09933	0.2288	1490	-0.434	0.9981	
POOZ - STZ	-0.39804	0.2508	1490	-1.587	0.6071	
SAZ - SIZ	-0.21350	0.0739	1490	-2.888	0.0453	*
SAZ - STF	-0.21541	0.1756	1490	-1.227	0.8238	
SAZ - STZ	-0.51412	0.2056	1490	-2.500	0.1245	
SIZ - STF	-0.00191	0.1858	1490	-0.010	1.0000	
SIZ - STZ	-0.30062	0.2120	1490	-1.418	0.7160	
STF - STZ	-0.29871	0.2693	1490	-1.109	0.8777	

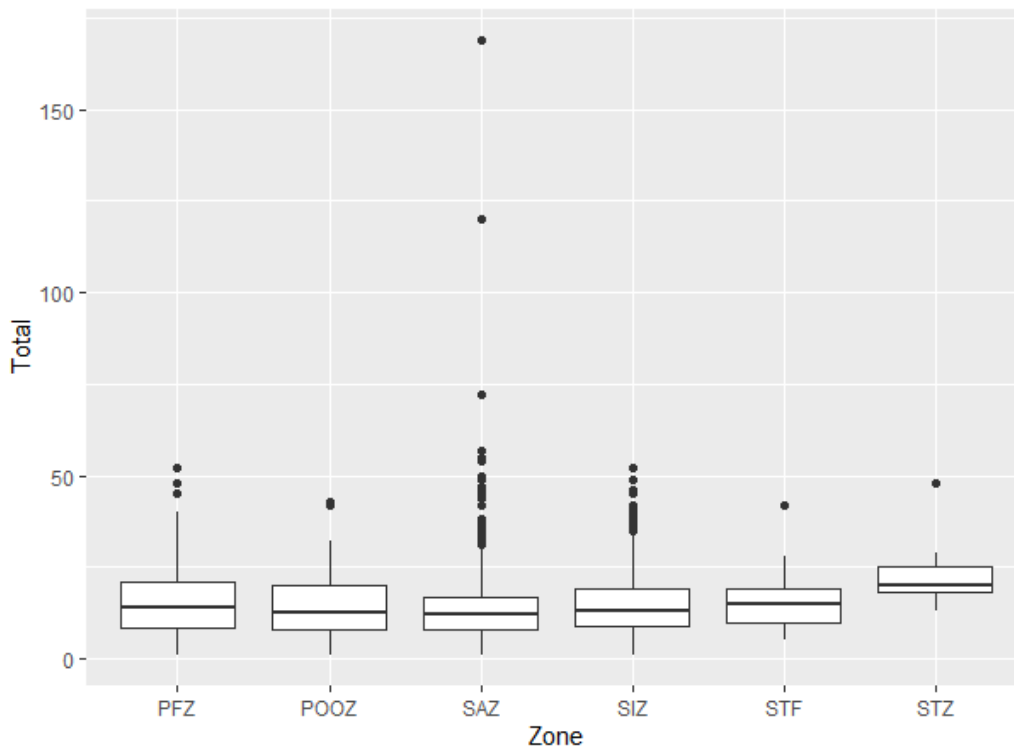


Figure 2.11: Distribution of synthetic particle abundances between five oceanographic zones (Polar Frontal Zone – PFZ, Permanent Open Ocean Zone – POOZ, Sub-Antarctic Zone – SAZ, Sea Ice Zone – SIZ, & Sub-Tropical Zone – STZ) and a frontal system (Sub-Tropical Front – STF), with data pooled across years, months, and transit.

## Particle characteristics

### Type of particle

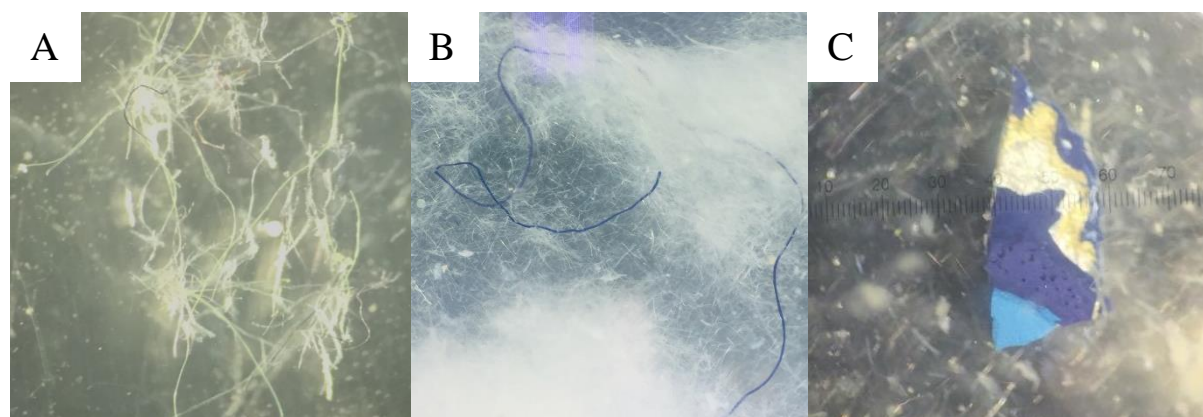


Figure 2.12: Examples of different synthetic particles found during the sample analysis conducted within this study, where A) is a large (> 2 – 5 mm) yellow fibre, B) is a large (> 2 – 5 mm) blue fibre, and C) is a medium-size (> 1 – 2 mm) multi-layered paint chip.

Five different types of synthetic particles were observed within the CPR surface water samples collected between 2009 – 2018, including those seen in Figure 2.12. Microfibers were the most prolific type across all years, accounting for between 90.6 – 99.5% of all observed microplastics (Figure 2.13). Fragments were also found throughout the span of the study, however these only accounted for between 0.8 – 8% of all microplastics. Synthetic particles such as foils, paint chips, and captured macroplastics (> 5 mm) comprised a negligible amount of the total particles found throughout the entire study. In support of this, the percentage of each particle type (fibres, fragments, and ‘other’) was shown to differ significantly ( $\chi^2(16) = 627.46$ ,  $p = <0.0001$ ).

Table 2.6: Count data investigating the number of synthetic particles collected between New Zealand and the Ross Sea Region, using CPR tows.

Year	Fibres	Fragments	Foils	Macro (> 5mm)	Paint
2009/10	3194	42	1	0	1
2010/11	2695	39	1	4	1
2011/12	344	12	0	0	0
2012/13	1108	107	6	0	2
2013/14	2565	129	3	0	0
2014/15	1449	29	1	0	1
2015/16	1268	11	3	5	1
2016/17	3537	17	0	0	0
2017/18	6328	30	0	13	0

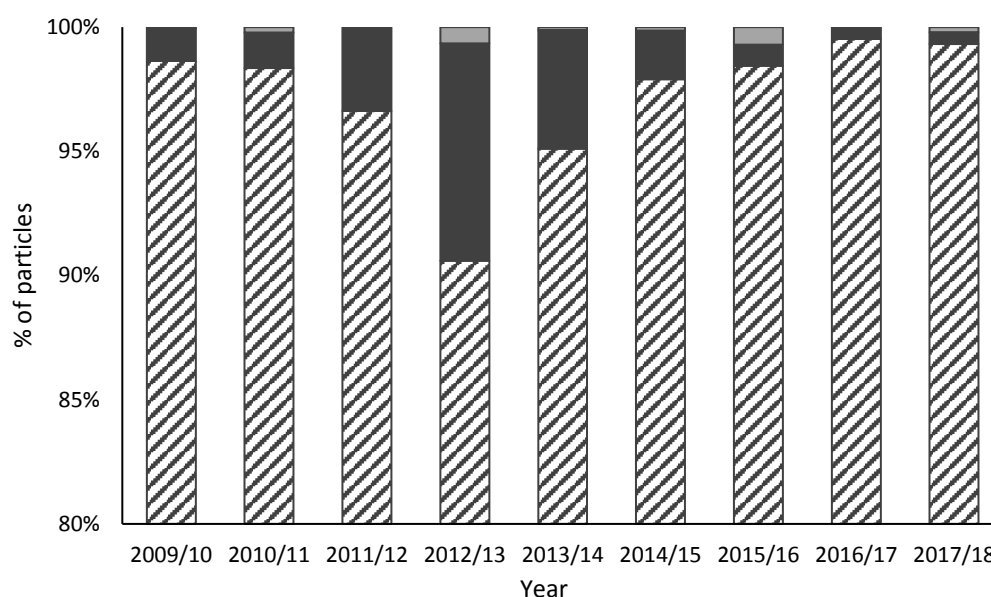


Figure 2.13: Percentages of the total count data between 2009 - 2018, for all different plastic types collected during CPR tows. Data is pooled across oceanographic zones. Striped bars represent the percentage of microfibres, black bars represent the percentage of fragments, and open bars represent the percentage of all other plastic types – foil, macroplastic, and paint chips.

### Size of particles

Throughout this study, 22,913 microplastic particles were found in the surface waters between New Zealand and the Ross Sea Region (small = 6062; medium = 8697; large = 8154), excluding any macroplastic particles (Table 2.7). In the first three years of sampling, large particles were the most predominant, accounting for up to 56.7% of all microplastics. However, the CPR tows between 2013/14 – 2017/18, the proportion of these dropped off and medium-sized particles became the most predominant size category (Figure 2.14). However, particle size categories were still found to be significantly different from one another ( $\chi^2$  (16) = 1871,  $p = <0.0001$ ).

Table 2.7: Count data investigating size distributions of synthetic particles in surface waters between New Zealand and the Ross Sea Region. Count data not inclusive of macroplastic particles >5 mm in length or diameter.

	Small (< 1 mm)	Medium (> 1 – 2 mm)	Large (> 2 – 5 mm)
2009-10	591	811	1834
2010-11	566	645	1532
2011-12	121	96	139
2012-13	489	372	354
2013-14	860	973	861
2014-15	373	691	414
2015-16	282	627	370
2016-17	877	1545	1132
2017-18	1903	2937	1518

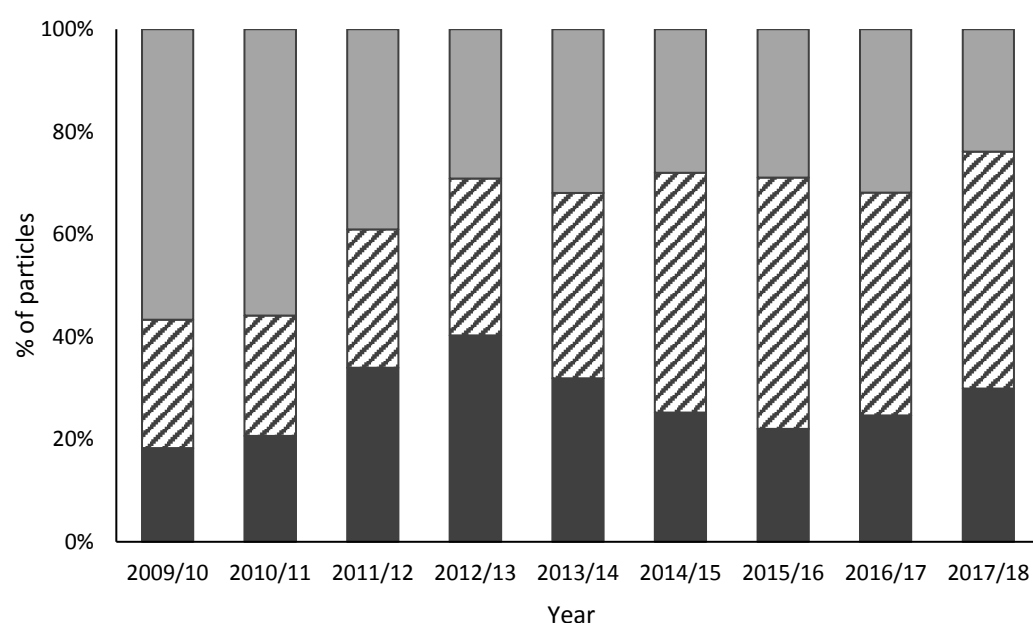


Figure 2.14 : Microplastic size distributions between 2009 – 2018, expressed as a stacked percentage. Black bars represent the percentage of small particles (< 1 mm), striped bars represent medium (> 1 – 2 mm), and grey bars represent large particles (> 2 – 5 mm).

### Colour of particles

Microplastics ranged in colour significantly throughout the surface water CPR samples, with nine different colours detected overall. Black and blue particles were the predominant colours found throughout the study (Figure 2.15), however other primary colours (red and yellow) were also highly abundant (Table 2.8). In support of this, the percentage of various colours analysed (black, blue, red, yellow, and ‘other’) was found to be significantly different ( $\chi^2$  (32) = 1298.5,  $p$  = <0.0001).

Table 2.8: Count data investigating colour distributions of synthetic particles in surface waters between New Zealand and the Ross Sea Region, between 2009 - 2018.

	Black	Blue	Colourless	Green	Orange	Purple	Red	White	Yellow
2009/10	1272	907	0	42	116	112	351	26	408
2010/11	1081	764	75	28	46	108	356	14	260
2011/12	149	131	0	10	9	3	42	5	7
2012/13	502	450	4	24	26	46	146	11	7
2013/14	1101	626	8	36	72	80	474	29	268
2014/15	632	366	2	9	25	76	262	9	98
2015/16	511	341	3	24	16	13	139	2	236
2016/17	1007	866	0	40	40	45	632	16	908
2017/18	2460	1701	0	58	43	67	1111	42	880

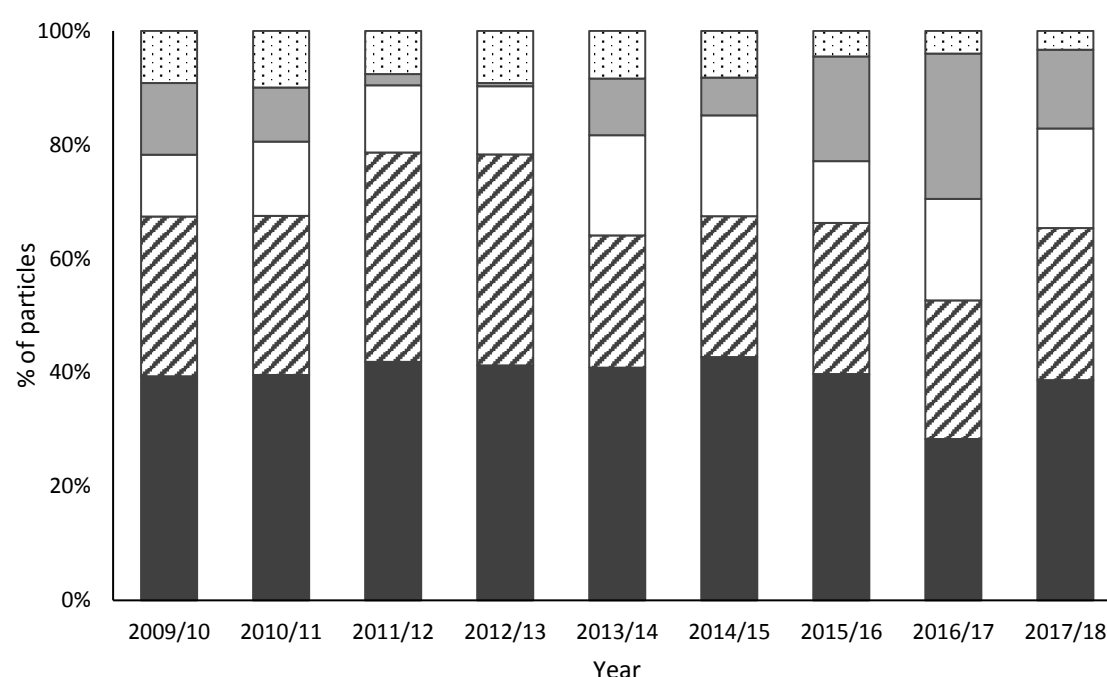


Figure 2.15: Microplastic colour distributions between 2009 – 2018, expressed as stacked percentages of the total count data for each year. Black bars represent the percentage of black particles, striped bars represent blue particles, open bars represent red particles, grey bars represent yellow particles, and dotted bars represent the total percentage of all other colours (colourless, green, orange, pink, purple, and white).

## Discussion

This study provided no evidence for consistent temporal or spatial patterns of microplastic occurrence between New Zealand and the Ross Sea Region of Antarctica, across the 9-year dataset. There was, however, a large amount of variation between years and zones, indicating that microplastic abundance and distribution is highly dynamic in these surface waters. In contrast to the initial hypothesis, the microplastic load within the sub-surface waters did not appear to increase with proximity to New Zealand, nor did the distribution change significantly across this 22,000 km stretch of the Southern Ocean. Despite microplastic levels not showing a clear increasing or decreasing temporal trend across the entire 9-year sample period, the mean abundance was highly dynamic and particularly for the tows collected during 2009/10 and 2012/13. The austral summer of 2009/10 contained up to double the average amount of microplastics compared to some other years, while those collected during the 2012/13 period had the smallest microplastic pollutant load overall. However, the morphotype most commonly found over the entire study was microfibrils, which supports the original hypothesis. The variation in both spatial and temporal components of this study are likely due to a combination of factors, particularly environmental drivers, as well as sampling error.

Despite a lack of consistency in spatial or temporal distributions of microplastic between 2009/10 – 2017/18, the morphotypes, size distributions, and colours of particles found throughout the sub-surface waters of the Southern Ocean was quite consistent. Black and blue coloured particles were of highest abundance throughout the study, whilst size of particles favoured the medium size distribution ( $> 1 - 2$  mm) in earlier surveys, then large microplastics ( $> 2 - 5$  mm) in later years. This could be due to sampling methodology, namely the aperture of the CPR mesh size, or alternatively, particles  $> 1 - 5$  mm in length/diameter may simply be more prevalent in these waters. However, throughout this entire study, microfibrils were by far the most prevalent morphotype of synthetic particle present, occupying between 90.6% - 99.5% of all synthetic particles collected each year. These results are relatively similar to the particle characteristics reported in other sea surface studies, particularly as microfibrils are thought to comprise up to 90% of the global marine microplastic floating debris (Barrows *et al.*, 2018; Woods *et al.*, 2018).

From 2017 onwards, there has been a considerable rise in the number of studies looking at marine plastic pollution in the Southern Hemisphere, particularly around the Antarctic continent. This surge in publications is likely due to the realisation that knowledge of the

abundance and distribution of marine plastic debris – particularly microplastics – had previously been limited to the Northern Hemisphere, thus creating a divide in the global knowledge of this pollutant. The recent research largely encompasses studies that have been confined to certain regions around the continent, where they have examined localised surface waters (Cincinelli *et al.*, 2017; Lacerda *et al.*, 2019), sediments (Munari *et al.*, 2017; Reed *et al.*, 2018), or the abundance and impacts of microplastics upon a single species (Dawson *et al.*, 2018; Bessa *et al.*, 2019). All of these examples have provided evidence for the presence of microplastics, however the estimated abundance differs significantly, largely as a result of the various sampling and analyses used. Inclusive of all samples between 2009/10 – 2017/18, regardless of whether they contained synthetic particles or not, there was an average of 1.99 particles km<sup>-1</sup>, of which ranged between 0.33 – 4.84 particles km<sup>-1</sup>. As a concentration, this load was extremely low, averaging 1.99e-6 particles per m<sup>3</sup>. In comparison, a paper examining near-shore and off-shore microplastic abundances in the Ross Sea reported between 0.0032 – 1.18 particles m<sup>-3</sup> through the use of a saltwater intake pump system (Cincinelli *et al.*, 2017), whilst an alternative study using manta nets to conduct surface trawls around the Antarctic Peninsula reported average concentrations of 0.008 particles m<sup>-3</sup>, although this was inclusive of both micro- and mesoplastics (>5 – 20 mm) (Lacerda *et al.*, 2019).

A lack of cohesive sampling methodology in the field of microplastic research provides another source of variation between results of different studies, making it extremely difficult to compare between studies in similar oceanographic regions. For the examination of surface waters, three different methods are commonly used to execute transects on or just below the sea surface – neuston nets, grab systems, and Continuous Plankton Recorders – all of which have different apertures, mesh sizes, and collection techniques. Most techniques used for sampling microplastics on the sea surface or sub-surface have been adapted from their original use of collecting plankton (Robinson *et al.*, 2014; Barrows *et al.*, 2017; Green *et al.*, 2018). Consequently, multiple factors such as mesh size and contamination influence the accuracy of the results gathered from each of these methods. Mesh size, for example, varies significantly across the literature, with many tows using a 333 µm mesh (Miller *et al.*, 2017), whilst CPRs use a 270 µm mesh (Robinson *et al.*, 2014) and others may use a different size altogether (Miller *et al.*, 2017; Green *et al.*, 2018). The size of the mesh often impacts the lower size limit you can collect, which is particularly unfavourable for examining the relative abundance of microplastics <1 mm. On the other hand, neuston nets and CPRs have the



advantage of sampling over large areas compared to grab systems, which are reliant upon sampling from the shore or a boat and collect small volumes of water (Barrows *et al.*, 2017). In saying that, further variability arises due to neuston nets conducting transects right at the sea surface, while CPRs are towed ~10 m beneath the surface. These examples are just some of the ways that the different methods of sea surface sampling differ, however they all contribute to a high variance in results and therefore a limited ability to effectively compare results between samples collected using different methodologies.

### Temporal variation

This study indicates that temporal changes to surface water microplastic abundance are likely driven by environmental factors which are extremely variable. As opposed to what was hypothesized, microplastic abundance did not increase between the austral summer of 2009/10 and 2017/18, despite global production of plastic materials continuing to grow (PlasticsEurope, 2019). One possibility is that this yearly variation is related to El Niño and La Niña events, as the two years with the highest average microplastic load (2009/10 & 2015/16) are also years that have experienced significant El Niño events (Welhouse *et al.*, 2016; Ministry for the Environment, 2017). On the other hand, La Niña events appear to correlate with years that have a more moderate average number of microplastics (11 – 14 particles per 5 m transect) and the lowest average microplastic load, which occurred in 2012/13, correlates to a neutral year with no obvious La Niña or El Niño trade winds. The El Niño-Southern Oscillation (ENSO) is a significant climate cycle that operates on decadal and sub-decadal periods (Welhouse *et al.*, 2016), with primary effects felt across the Pacific Ocean (Turner, 2004). However, effects of the phenomenon have been documented in many other regions of the globe, including Antarctica. El Niño events are thought to account for an estimated 31% of the altered sea surface temperature conditions, along with higher pressure areas in the West Antarctic (Welhouse *et al.*, 2016). La Niña events, on the other hand, are reportedly associated with negative temperature anomalies around the Ross Sea (Welhouse *et al.*, 2016). In addition to changes in temperature, El Niño and La Niña events can alter other aspects of the Antarctic climate, including winds and ocean currents. As reported by Kim & Orsi (2014), the ACC fronts tend to respond to both ENSO and SAM (the Southern Hemisphere annular mode), with localised variations in features such as surface wave height – possibly resulting in increased mixing of synthetic particles. Furthermore, ENSO and SAM variation has been widely documented to result in significant oceanographic changes throughout the ACC system, with positive SAM and La Niña events driving polar lows and

forcing upwelling, whilst El Niño and negative SAM causing the opposite; fewer storms and a reduction in upwelling (Marshall *et al.*, 2004; Yuan, 2004; Martinson, 2012). Wide-scale alterations such as these are likely to have some impact upon the circulation of microplastics throughout the Southern Ocean, with rates of deposition and typical patterns of dispersal likely interrupted. Interestingly, anomalies in ENSO events throughout this sampling period seem to correlate with the years that experienced the highest and lowest microplastic loads in the sub-surface waters, respectively. These climate events are not likely to be the only environmental drivers of yearly variation in microplastic abundance, but they do indicate that further investigations into similar large-scale oceanic circulation trends are warranted.

As hypothesized, monthly variation between average microplastic occurrence was minimal. This was to be expected, as sampling could only occur during the Austral Summer months of each year – primarily between November and February. To gain a more complete perspective of the monthly variation for surface water microplastic abundance, future studies would ideally need to examine the abundance across all 12 months. This would provide information on whether there are any significant temporal differences in microplastic abundance on a smaller scale. So far, numerous polar studies in the Arctic and Antarctic have illustrated the ability of sea ice to take up high concentrations of microplastics during the winter months (Obbard, *et al.*, 2014; Peeken *et al.*, 2018; Geilfus *et al.*, 2019). In addition, microplastic load is likely to change throughout the year with the contributing factor of winter storms, and the resultant deepening of the surface mixing layer (Nicholson *et al.*, 2016). Although studies have not yet focussed on the change in microplastic abundance within Antarctic waters throughout all 12 months – likely due to the logistical difficulties in carrying out sea-based research during the Antarctic winter and the high frequency of winter storms – it is a reasonable assumption that microplastic loads in Antarctic waters may experience high levels of variability throughout the year.

### Spatial variation

In a similar vein to the temporal variation observed within this study, there are no clear increasing or decreasing trends regarding the levels of microplastic abundance between the different oceanographic zones. Instead, microplastic abundance was found to be relatively similar in its distribution across most of the oceanographic zones, which differed to the results reported in Suaria *et al.* (2020). This study collected 40 neuston net samples from the surface waters of Antarctica, during a circumnavigation of the continent. Although the primary focus was not to analyse the differences in microplastics between various latitudinal

zones, their results suggested that microplastic abundance was an order of magnitude lower in latitudinal zones below the Sub-Tropical Front (STF), where this front acts like a boundary. The results from this thesis directly contradict this conclusion, as the long, continuous transects executed between oceanographic zones detected no significant difference in abundance immediately above or below the STF. This contrasts with the original hypothesis, indicating that proximity to an urbanised nation such as New Zealand does not have a strong impact upon microplastic distribution. Several factors may be driving these results, particularly environmental variables such as oceanic currents. The land mass of Antarctica may be geographically isolated from all other continents, but the Antarctic Circumpolar Current (ACC) connects the Southern Ocean with all other major oceanic systems. Consequently, it is likely that the currents which circulate around the continent also bring synthetic particles to the area, as microplastics are typically buoyant with the ability to undergo long periods of dispersal (Obbard, 2018). Studies such as Obbard (2018) suggest that the incidence of man-made microplastics in polar waters is primarily the result of long-range oceanic transport through global current systems, waves, and wind, as opposed to local inputs from production and human usage. This long-range transport to remote polar regions generally enhances the occurrence of microparticles instead of larger debris, as these smaller items are thought to be submerged within the sub-surface oceanic layer during transport, consequently taking longer to reach the polar environments, simultaneously being subjected to fragmentation and degradation as well (Lebreton & Borrero, 2013; Desforges *et al.*, 2014; Obbard, 2018). Therefore, the Antarctic Circumpolar Current not only transports microplastics to the Southern Ocean but is also responsible for mixing of these synthetic particles through the three main oceanic fronts, which are further described in Orsi *et al.* (1995) and Solokov & Rintoul (2009).

In conjunction with occupying numerous oceanographic zones, the Southern Ocean provides a habitat for a unique ecosystem, with many endemic species that have adapted to the harsh environment. Overall, this study provides evidence for microplastics occurring within the surface waters of every zone between New Zealand and the Ross Sea. Consequently, species that carry out functional roles within the sub-surface of the water column are at a higher risk of direct interactions with these synthetic particles, particularly if they feed within this part of the water column and are filter feeders. *Euphausia Superba* (Antarctic Krill) are one such species, notable in the Southern Ocean for occupying a keystone role in the ecosystem, comprising the main dietary component of higher trophic levels such as whales and penguins,

for example (Laws, 1977; Bernard & Steinberg, 2013). This species has an alternating seasonal feeding regime, primarily consuming phytoplankton in the summer months and a range of zooplankton and copepods come winter (Price *et al.*, 1988; Bernard & Steinberg, 2013). However, in conjunction with their prey items, *E. superba* have been shown to ingest microplastic particles from the water column, then expel most of these particles as smaller nanoplastics (<1 µm) (Dawson *et al.*, 2018). Since Antarctic krill are an integral prey item for many other Antarctic marine species, the uptake of synthetic particles increases the likelihood of microplastics being passed up the food web. On the other hand, the excretion of plastic nanoparticles may provide this pollutant with new ways to enter the food web, such as ingestion by zooplankton and the ability to cross physical barriers (Dawson *et al.*, 2018). In addition, the current literature demonstrates that the sea surface is not the end point for microplastics in the marine environment; instead they drift down to the benthos (Munari *et al.*, 2017; Reed *et al.*, 2018), interact with biota (Bessa *et al.*, 2019; Sfriso *et al.*, 2020), or in the case of polar regions, also incorporate into sea ice (Obbard *et al.*, 2014; Kelly *et al.*, 2020). Consequently, the presence of microplastics within the sea surface indicates that it is likely to be found elsewhere in the Antarctic marine environment, with the possibility for wide-reaching implications upon the entirety of the Southern Ocean's ecosystem.

## Conclusion

Although definitive trends in microplastic load were not detected in this long-term temporal and spatial study between New Zealand waters and the Ross Sea Region of New Zealand, these results have illustrated that microplastics are, indeed, present in these remote waters – likely as a result of long-range oceanic transport. The quantity of surface water microplastics is shown to be lower than that in other regions of the world, but synthetic particles were found throughout all sampled years (2009/10 – 2017/18), as well as in every oceanographic zone between New Zealand and the Ross Sea Region. This is the first multi-year microplastic study to have been carried out in the Southern Hemisphere, and the first in this geographical region to examine latitudinal spatial distribution of the synthetic particles to this extent. Despite the need for further research to support these findings using FTIR spectroscopy, this study has provided an essential baseline for understanding the occurrence and dispersal of floating microplastic debris in a remote area of the globe.

### 3. Temporal surveys of microplastic load in sediment, surface water, and mussel (*Mytilus galloprovincialis*) tissue in Wellington Harbour

#### *Abstract*

This study aimed to take a multi-pronged approach to microplastic assessment in Wellington Harbour, through undertaking temporal and fine-scale spatial microplastic surveys on the sea surface, beach sediments, and within the tissues of *Mytilus galloprovincialis* mussels.

A series of baseline surveys were conducted every three months between February 2019 and February 2020. Beach sediments and *M. galloprovincialis* individuals were each collected from three sites around Wellington Harbour, with collections increasing to weekly surveys from August 2019, to assess temporal variation on a finer scale. Sea surface tows were conducted once every three months throughout the year, at two different locations within the harbour. The total microplastic abundance in each sample was recorded, along with size, colour, and morphotype of the synthetic particles.

Mussel tissues contained an average of  $0.30 \pm 0.04$  particles  $\text{g}^{-1}$  (wet weight) and were less temporally and spatially variable than beach sediments, which had an average of  $124.9 \pm 35.7$  particles  $\text{kg}^{-1}$  (wet weight). Spatial variation was observed throughout beach sediments, although differences among sites did not follow a consistent pattern throughout the span of the study. Surface water microplastic load appeared to correlate with the temporal peaks in mussel tissue, indicating a possible relationship between levels in the environment and the ability for transferral to coastal marine organisms. Microfibres were the predominant particle morphotype found within every source, whilst microplastic size was distributed far more evenly throughout the three categories. These responses suggest that microplastics are, indeed, a globally pervasive pollutant which infiltrate numerous marine habitats and interact with organisms.

## Introduction

In New Zealand, the ocean is central to daily life, with many individuals earning a living from it, as well as participating in countless recreational activities around the coastline. With an estimated 18,000 km of coastline in various forms that include open coast, harbours, estuaries, and lagoons (Hart & Bryan, 2008), New Zealand's society is unequivocally linked to, and surrounded by, the marine environment, and the coastal ecosystem is the primary interface between marine and terrestrial habitats. This results in the ecosystem being subject to many different processes, and consequently providing a pathway for pollutants like plastics to interchange between land and oceanic environments.

Research on microplastic load in New Zealand, including its abundance, distribution, and effects upon marine and intertidal species, is limited. Although some of the very first papers to ever document microplastic occurrence were carried out in New Zealand during the 1970's (Gregory, 1977; Gregory, 1978), little progress has been made since then. These initial studies surveyed a range of beaches around the New Zealand coastline, particularly within the Auckland, Wellington, and Canterbury regions, and provided some of the first direct evidence for microplastic contamination in the marine environment. Almost 40 years later, two more publications focussing on the occurrence of microplastics around New Zealand's coastlines. Clunies-Ross *et al.* (2016) was able to quantify both primary and secondary sources of microplastic pollution within Canterbury, whilst Bridson *et al.* (2020) focussed upon the microplastic load on Auckland beaches. All four of these New Zealand microplastic research examples published to date have placed a primary focus on beach sediments, as opposed to the abundance within other habitats or marine biota. Beaches are considered to be sinks for oceanic plastic pollution, particularly microplastics, as onshore winds push the buoyant particles onto the shore, dropping them at the high tide line (Moore, 2008; Clunies-Ross *et al.*, 2016). Consequently, the top layers of beach sediment provide a valuable insight into the abundance of microplastic particles that inhabit the surrounding waters. Microplastics present a unique problem once on the shoreline, however, as unlike macroplastic, they are often difficult to distinguish in size from sand grains and therefore become easily incorporated into the beach sediments (Young & Elliott, 2016) (Figure 3.1).

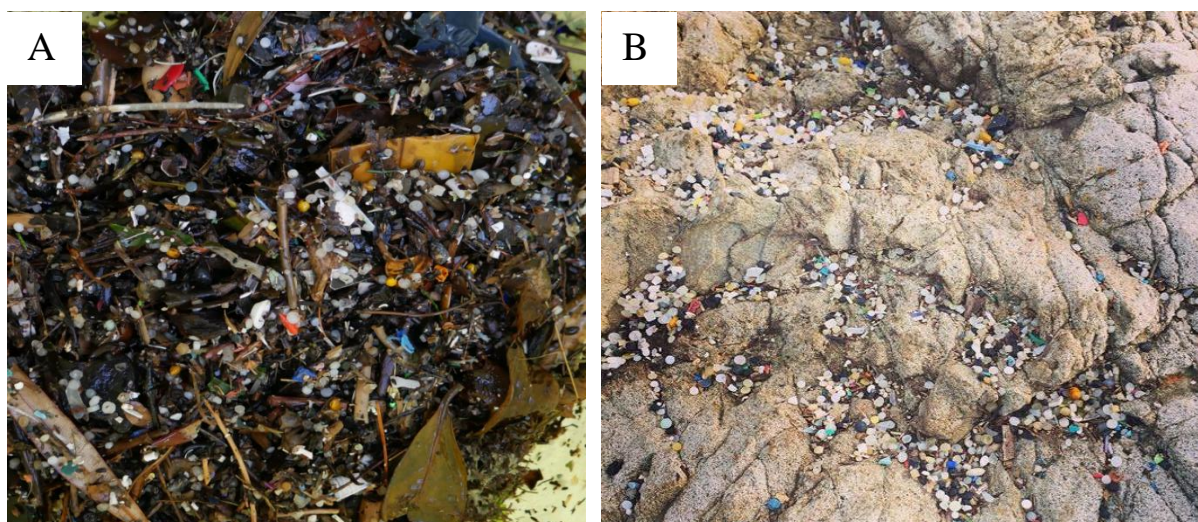


Figure 3.1: Microplastics found on Evan’s Bay Beach, Wellington, where A) depicts the difficulty of extracting synthetic particles from organic matter and B) demonstrates the occurrence of microplastics *in situ*. Image credit: A) Sea Shepherd New Zealand, B) Caitlyn Shannon.

Consequently, although it is still limited, our knowledge of microplastics on beaches far surpasses the current understanding of surrounding environments like sea surface waters or the pollutant load within coastal marine organisms. This extension to other habitats, along with an expansion of knowledge throughout various regions around the country, is integral if comparisons to the global microplastic load are to be made. Presently, there is only one paper which examines the occurrence of microplastics within New Zealand coastal organisms. Webb *et al.* (2019) reported an abundance of between 0 – 1.5 particles per individual within the *Perna canaliculus* (Green-lipped mussel) species, around the coastline of New Zealand. With exception to this paper, the only other knowledge of microplastic occurrence and impacts of microplastics upon our coastal environment has examined the freshwater inputs from urban rivers and streams (Dikareva & Simon, 2019; Mora-Teddy & Matthaei, 2020). With such a limited grasp upon microplastic abundance, distribution, and its impacts on the New Zealand ecosystem, comparisons to the global pollutant load cannot be made at this time.

Globally, surface waters are commonly associated with plastic debris of all size classes. Estimates of floating plastic pollution provide a valuable insight to the levels of plastic pollution permeating into other marine environments and biota, as the buoyancy of the pollutant means it often circulates at the surface of the water column before undergoing vertical mixing (Ye & Andrady, 1991; Morishige *et al.*, 2007; Maes *et al.*, 2017). Research examining the extent of floating plastic debris – both macro and micro – has been carried out for decades using many different sampling methodologies. One recent study has even evaluated the presence of floating plastic debris over the span of 60 years, to investigate



temporal trends over an extended period (Ostle *et al.*, 2019). However, like most research on marine plastic pollution, most of the work has been typically carried out in the Northern Hemisphere. Studies examining abundance and distribution on the sea surface in the Southern Hemisphere are limited (Avio *et al.*, 2017); for example there have been a few studies carried out in South Africa (Ryan, 1988; Nel & Froneman, 2015), Australia (Reisser *et al.*, 2013; Hall *et al.*, 2015), and Brazil (Lima *et al.*, 2014), as well as the South Pacific subtropical gyre (Eriksen *et al.*, 2013). Yet, these studies are integral to forming a more comprehensive understanding of how microplastics move throughout the oceans.

The occurrence of microplastics within marine organisms is another key facet of plastic pollution research. In contrast to the identification of microplastics in beach sediments and throughout the sea surface, research upon organisms elevates the field to a deeper level of understanding. Such studies promote a widening of knowledge from the rudimentary understanding that this pollutant is found within our marine environments, to an awareness that it is also infiltrating and impacting biological communities, and therefore conceivably also interacting with humans. The current global estimates of microplastics in the marine environment certainly pose a significant problem in themselves, as the high volume likely increases the possible interactions they have with the surrounding ecosystem for many years to come, but these issues are exacerbated with the current knowledge that the particles are able to enter the food web and cause physiological, behavioural, and structural difficulties for various marine organisms including invertebrates (Besseling *et al.*, 2013; Wright *et al.*, 2013; Galloway & Lewis, 2016) and fish (Critchell & Hoogenboom, 2018).

Bivalves such as mussels are useful bioindicators for assessing microplastic occurrence in coastal marine ecosystems, as well as indicating the level of transfer between the surrounding environment and marine biota. In addition, bivalves are filter-feeders with a global distribution (Chae & An, 2020). Suspension and filter-feeders are vulnerable to microplastic ingestion, as they continually filter larger volumes of water and are generally limited in their ability to selectively ingest particles (Kinjo *et al.*, 2019). Filter-feeders also tend to feed upon organisms that occupy a similar size range to microplastics, making it even more difficult to discriminate between food and synthetic particles. In fact, one study has demonstrated that when mussels are simultaneously exposed to alga and microplastics, the organisms are more likely to retain the plastic particles for longer before excreting them, compared to when they were exposed to microplastic without the presence of alga (Chae & An, 2020). Consequently,



filter feeders like mussels may be more susceptible to retaining microplastics for longer periods when there is also a high volume of food sources in the surrounding environment.

In New Zealand, three mussel species occupy the intertidal and shallow subtidal zones. The ribbed mussel (*Aulacomya maoriana*), the green-lipped mussel (*Perna canaliculus*), and the Mediterranean blue mussel (*Mytilus galloprovincialis*) co-exist in many different hard-shore environments along New Zealand's coastline, where they have similar ecological functions as primary consumers and sessile filter-feeders (Gardner, 2002). The New Zealand coastline provides an interface between the marine and terrestrial environment, so is often highly urbanised. Consequently, the coastal ecosystem experiences significant pollution from sources such as plastic, heavy metals, and sedimentation – to name a few (Vikas & Dwarakish, 2015). Coastal-dwelling species such as mussels are subjected to these pollutants on a regular basis, thus placed under significant stress and the possibility of trophic transfer throughout the food web. *Mytilus galloprovincialis* mussels are a particularly good model species for the investigation of microplastic occurrence, as they have a global distribution, meaning that there are comparisons that can be made from other studies. Additionally, as Li *et al.* (2019) states, mussels are likely to be a significant source of microplastic transferral to humans, as they are a common seafood that is typically consumed whole.

Temporal and spatial studies allow us to better elucidate the full extent of microplastic load in the marine environment, however currently, studies that consider the impacts of space and time are not commonplace. A study conducted by Baechler *et al.* (2020) has reported a difference in microplastic abundance within Pacific oysters (*Crassostrea gigas*) and Pacific razor clams (*Siliqua patula*) across different seasons, at different sites across the Oregon coastline. Consequently, we can see that attempts are being made to characterise spatial and temporal trends in microplastic load within the Northern Hemisphere, so it is valuable to undertake similar studies throughout coastal environments in the Southern Hemisphere – enabling comparisons to be made for future mitigation efforts.

Wellington Harbour is a semi-enclosed harbour composed of a roughly circular shape at the southern-most tip of the North Island, New Zealand (Kröger *et al.*, 2006). It has a total area of approximately 85 km<sup>2</sup> (Booth, 1975) and a benthos made up of soft sediment, particularly in the deeper parts of the harbour (Van der Linden, 1967). Towards the harbour entrance and around nearshore areas, however, the sediment layer is reported to be coarser (Carter, 1977; Kröger *et al.*, 2006). A relatively shallow harbour, the average depth is 14 m, but it reaches

32 m South of Matiu-Somes Island (Heath, 1977; Kröger *et al.*, 2006) and is considered well-mixed (Kröger *et al.*, 2006). Its main source of fresh water is the Hutt River, which itself is subject to relatively high levels of pollution of various kinds (Booth, 1975), including inputs of plastic pollution (Mora-Teddy & Matthaei, 2020). As a heavily urbanised harbour with significant man-made alterations, it is likely that there are high levels of microplastic pollution in all aspects of the habitat – whether that be the sea surface, beach sediments, or in the tissues of coastal organisms that inhabit Wellington Harbour.

This study aimed to provide the first quantitative baseline survey of microplastic debris in Wellington Harbour, using a multi-pronged approach. An emphasis was placed upon collecting data from multiple sources, including surface water, the shoreline, and the tissues of *Mytilus galloprovincialis*, for the purpose of addressing the following questions: does larger-scale (3-monthly) and shorter-scale (weekly) temporal variation play a role in determining microplastic abundance within Wellington Harbour? Do all three sources show coherent patterns of microplastic abundance and distribution within the harbour? Finally, is the level of microplastic pollution found in Wellington's surface waters, beaches, and mussels similar to abundances found elsewhere in New Zealand and the Southern Hemisphere?

## Methods

### Sample collection

Samples were collected between the 7<sup>th</sup> of February 2019 and the 15<sup>th</sup> of February 2020, from a total of three beach sites, three mussel collection sites, and two surface water tow sites. Samples were collected every three months for a year, with shoreline and mussel surveys occurring in February, May, August, and November, while surface water tows occurred in March, June, September, and December. For the first two sediment and mussel collections (February & May 2019), only one sampling event took place at each site per month. To better elucidate variability over shorter time scales, samples were collected once a week for three weeks at each beach and mussel collection site in August (2019), November (2019), and February (2020). During all field collections, non-synthetic clothing was worn, so to minimise contamination.

### Beach sampling

Beach samples were collected during low tide from Oriental Bay, Balaena Bay, and Seatoun Beach (Figure 3.2). Three replicate samples were collected at what was visually determined as the most recent high tide line, which is known to be the area on a shoreline to accumulate the highest amount of plastic debris (Hidalgo-Ruz *et al.*, 2012). To ensure sampling was unbiased, samples were taken at a random distance apart. This was determined by throwing a sediment corer (10 cm diameter) and collecting sediment from the point on the high tide line where it landed. The corer was marked with centimetre notches, and the first 5 cm of sediment were collected. The total sediment volume collected for each sample was  $\sim 400 \text{ cm}^3$  ( $V = \pi 5^2 5$ ). Samples were returned to the laboratory for analysis.

### Mussel sampling

Individual mussels (*Mytilus galloprovincialis*) were collected at low tide from rocky substrata at three sites around the Wellington Harbour, all with high mussel abundance; Oriental Bay, Kau Point, and Scorching Bay (Figure 3.2). At each location, 5 mussels with a shell length of  $\geq 30 \text{ mm}$  (measured with calipers to the nearest 0.1 mm) were collected from the substrate and placed in a small bag, before being returned to the laboratory for processing.

### Surface water sampling

Small boats belonging to the Victoria University Coastal Ecology Laboratory (VUCEL) were used to collect plankton along two 200 m long transects: one from the South-West of Mātiu-Somes Island (S 41°15, E 174°51), in the middle of Wellington Harbour, and one from the mouth of Evans Bay (S 41°17 E 174°49) (Figure 3.2). The vessel cruised at a speed of  $\sim 5$

knots during the collection, while a neuston net was towed behind it. Seawater was filtered through a 250 µm mesh collecting sieve, with organic and inorganic material collected in a collecting tube. After the transect had been completed, the material in the collecting tube was transferred into a bucket and the tube was rinsed to collect all organisms and particles. Samples were then taken back to VUCEL for further filtering and laboratory analysis. The tows were carried out using a neuston net, which is traditionally used to capture plankton, but also commonly used for obtaining surface water microplastics (Barrows *et al.*, 2017; Green *et al.*, 2018). Due to logistical limitations, only one transect was carried out at each of the two sites every three months – these occurred on the same day. To minimise contamination between the two sites, the net and collecting mesh were both rinsed with fresh water before the commencement of the tow at the second site (Löder & Gerds, 2015).

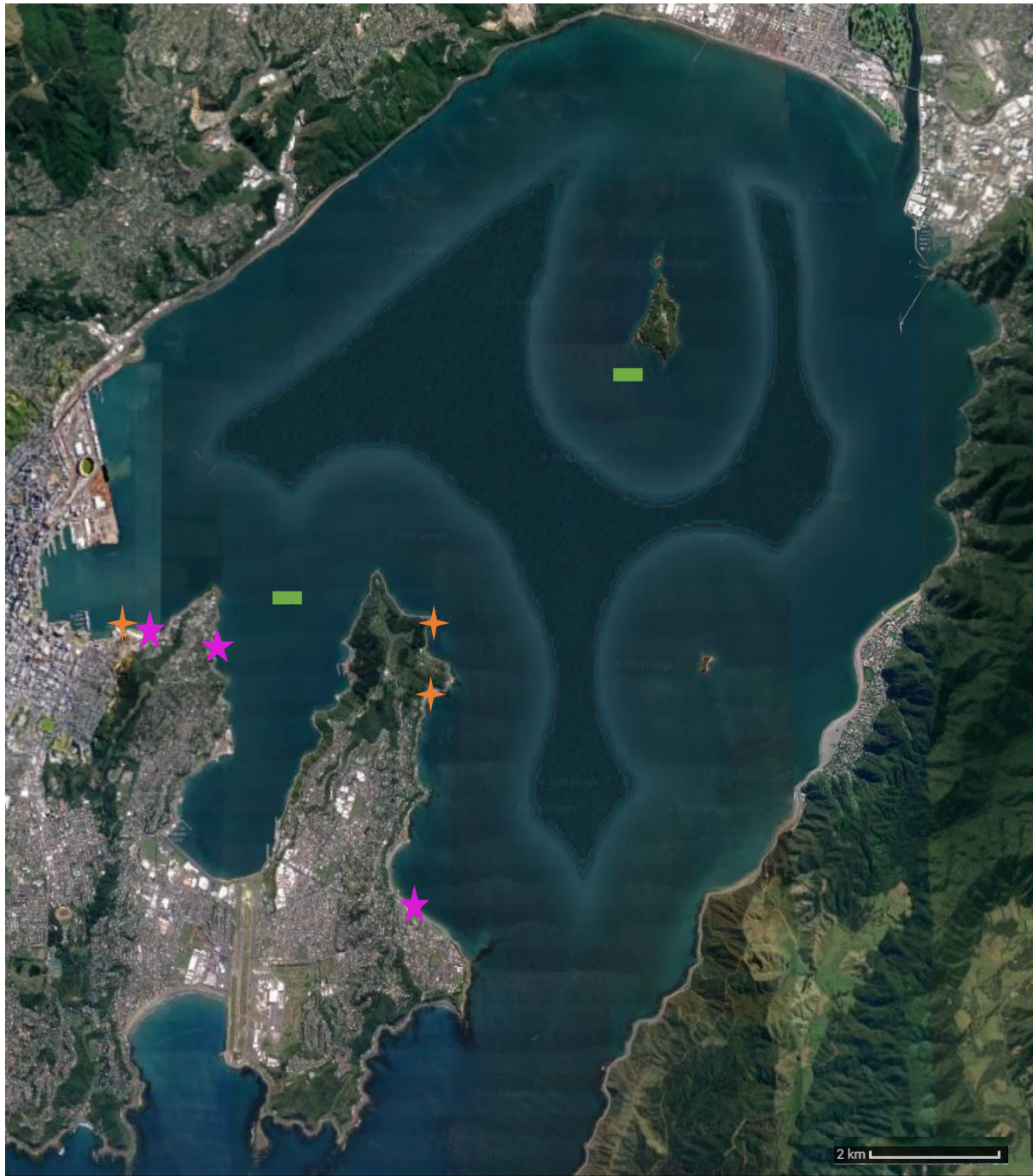


Figure 3.2: Map of sites sampled within Wellington Harbour, where pink symbols represent beach sediment survey sites, orange represents *M. galloprovincialis* mussel sites, and green demonstrates surface water transects. Retrieved from Google Earth 21/05/2020.

## Laboratory analysis

### Beach samples

Once in the laboratory, a density-separation method was used to extract any synthetic particles from the sediment collected in the field. This method was adapted from techniques first used by Thompson *et al.* (2004) and Claessens *et al.* (2011). For every 200 g of sediment, 400 mL of super-saline NaCl solution was added. Beakers were then manually stirred and shaken for 30 seconds (Clunies-Ross *et al.*, 2016), and then the contents allowed to settle for 1 minute. The top layer of debris was then siphoned off and filtered through a 75 µm sieve. This process of manually stirring, settling, then filtering was repeated until there was only a thin layer of NaCl solution left above the sediment (1-2 mm), with the leftover sediment then discarded. The process was repeated until all the sediment from each sample had been density-separated and filtered.

### Mussel samples

After field collection, the mussels were returned to the laboratory and placed in the freezer for 48 hours. 1 hour before processing, they were removed from the freezer to thaw out. At this time, shell length was confirmed using calipers and they were placed in individual jars. To minimise contamination from particles in the air, each jar was covered in cling wrap during thawing and digestion. After the mussels had thawed out sufficiently, the flesh was removed from the shells and weighed at wet weight .

To prepare for mussel digestion, a 10% KOH solution was made, using KOH pellets and distilled water in a 1L flask, following an adapted protocol described in Miller *et al.* (2017), used in Tanaka & Takada (2016) and Wagner *et al.* (2017). The 10% KOH solution was poured into each jar until the mussel tissues were completely covered (Figure 3.3). At this stage, the cling wrap was added, and the tissues were left at room temperature for a total of 2 weeks, stirring every few days. After two weeks, all *M. galloprovincialis* tissue had been digested, leaving only inorganic particles. To remove these and analyse them, the KOH solution was filtered through a 75 µm sieve, and the collected particles rinsed with distilled water before being transferred to a petri dish for examination under the microscope.



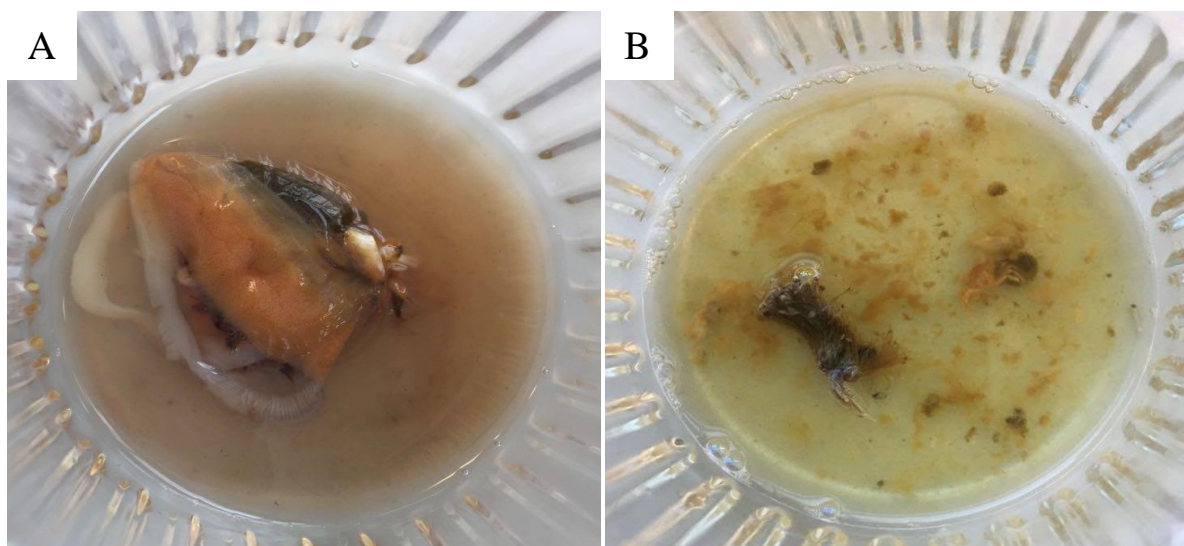


Figure 3.3: Mussel digestion process, including A) at the commencement of digestion, as the 10% KOH solution was being added, and B) after a week of digestion, where there was only a small amount of soft tissue left, along with byssal threads and inorganic materials. At the end of the digestion process, there was no soft tissue left, and the byssal threads had dispersed.

### Water surface samples

Once in the laboratory, each sample was filtered further through a 100  $\mu\text{m}$  sieve, again using the adapted density-separation and filtering methodology first described in Thompson *et al.* (2004) and Claessens *et al.* (2011). A siphon was again used to collect particles and filter them, before the particles were transferred to a petri dish using distilled water. These samples were then ready for examination under the microscope.

### Microscope analysis

Once filtered, each sample was transferred to a petri dish, using distilled water. These were then visually examined under a stereo microscope at 15x magnification. Items were characterised in the same way as the previous data chapter, with three size classes – small (<1 mm), medium (1-2 mm), and large (2-5 mm). Type of particle was also recorded, with categories for fibres, fragments, foil, paint, and macroplastic. Additionally, the colour of each particle was noted. Items were verified as synthetic by attempting to break apart the item with forceps, as well as the visual analysis. If the object was plastic, it would not break under pressure of the forceps. After examination, samples were again rinsed through the 75  $\mu\text{m}$  filter then transferred to a collection tube and preserved with 40% ethanol.

### Statistical analyses

All analyses were conducted in R V3.6.3 (R Core Team 2020) using RStudio v1.2.5033 (RStudio team, 2020). Pearson's Chi-squared tests were used to test morphotype, size, and

colour of synthetic particles collected during the study. Additionally, a generalized linear model (GLM) with a Poisson distribution was used to test for temporal and spatial variation within the *M. galloprovincialis* tissue samples and beach sediment samples, due to non-normal distribution of count data. Tests were conducted for temporal variation, both between months (February 2019 – February 2020), and between weeks for the applicable collections that occurred during August 2019 – February 2020. Tests for spatial variation were conducted for the three different sites within a collection month – Oriental Bay (OB), Kau Point (KP), and Scorching Bay (ScB) for mussels, and Oriental Bay (OB), Balaena Bay (BB), and Seatoun Beach (ST) for beach sediments. Results were reported on a log scale.

Finally, due to a small sample size ( $n = 8$ ) and non-normal distribution, a Kruskal-Wallis test was conducted to quantify temporal differences between the surface water tows, using the two sites (Evans Bay & Matiu-Somes Island) as the two replicates.



## Results

### *Mytilus galloprovincialis*

#### Monthly variation

Mean microplastic abundance within *M. galloprovincialis* individuals was highly variable, dependent upon the month of sampling. February 2019 was significantly different to all other months (Table 3.1), with values approximately 5 times higher than May 2019, and up to 13 times greater than all other sampled months (Figure 3.4). In addition, there was also a significant difference between May 2019 and all subsequent months (Table 3.1), with mean microplastic abundance was twice as high (Figure 3.4).

Table 3.1 Results of between-month variation (February 2019, May 2019, August 2019, November 2019, and February 2020) on microplastic abundance within pooled *M. galloprovincialis* samples, where \* indicates significant differences at  $p = 0.05$ .

Month	Estimate	SE	Z.ratio	P.value	
Aug19 - Feb19	-2.4155	0.102	-23.790	<0.0001	*
Aug19 - Feb20	-0.0081	0.127	-0.064	1.0000	
Aug19 - May19	-0.6039	0.147	-4.122	0.0004	*
Aug19 - Nov19	0.2375	0.136	1.749	0.4039	
Feb19 - Feb20	2.4074	0.101	23.786	<0.0001	*
Feb19 - May19	1.8116	0.125	14.545	<0.0001	*
Feb19 - Nov19	2.6530	0.112	23.740	<0.0001	*
Feb20 - May19	-0.5958	0.146	-4.073	0.0004	*
Feb20 - Nov19	0.2456	0.136	1.812	0.3667	
May19 - Nov19	0.8414	0.154	5.472	<0.0001	*

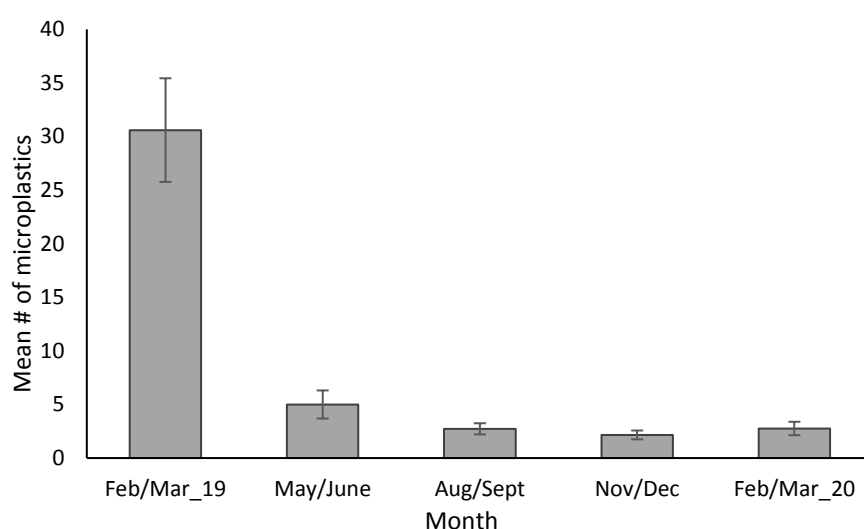


Figure 3.4: Mean (+/- 95% confidence interval) number of microplastics observed within *M. galloprovincialis* samples collected in February 2019, May 2019, August 2019, November 2019, and February 2020.

## Weekly variation

*M. galloprovincialis* tissues had a statistically similar microplastic load throughout the three collections in August 2019 and November 2019 (Table 3.2). In contrast, the third week of sampling in February 2020 was significantly different to week 1 and 2 (Table 3.2), with *M. galloprovincialis* mussels containing approximately half the amount of microplastics observed in the previous weeks within their tissues (Figure 3.5).

Table 3.2: Results of a pairwise comparison, showing between-week variation of microplastic abundance within *M. galloprovincialis* samples, where \* indicates significant differences at  $p = 0.05$ . Collections A – C represent weeks 1 – 3, respectively.

Month	Collection	Estimate	SE	Z.ratio	P.value	
Aug-19	A - B	0.2029	0.213	0.952	0.6071	
	A - C	0.3655	0.223	1.637	0.2299	
	B - C	0.1625	0.233	0.697	0.7654	
Nov-19	A - B	0.2162	0.25	0.867	0.6614	
	A - C	0.1178	0.243	0.485	0.8785	
	B - C	-0.0984	0.256	-0.384	0.9220	
Feb-20	A - B	0.2007	0.201	0.998	0.5779	
	A - C	0.8293	0.245	3.390	0.0020	*
	B - C	0.6286	0.253	2.487	0.0344	*

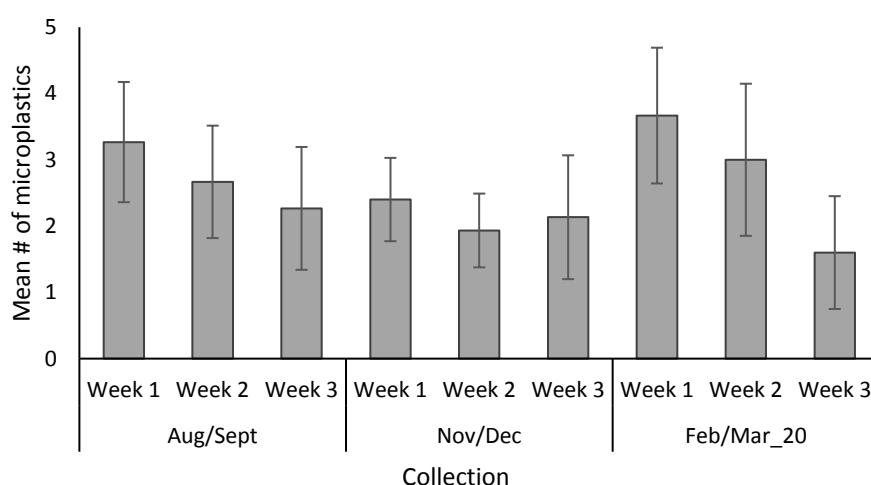


Figure 3.5: Mean (+/- 95% confidence interval) number of microplastic particles within *M. galloprovincialis* tissues, with variation observed between weekly collections in Aug/Sept '19, Nov/Dec '19, and Feb/Mar '20.

### Spatial variation

For *M. galloprovincialis* samples, the monthly mean microplastic abundance was not heavily influenced by site. The only significant differences between site occurred within the month of August 2019, where the mean microplastic abundance differed between Kau Point and Oriental Bay individuals (Table 3.3), with mean microplastic abundance greater in individuals collected from Oriental Bay (Figure 3.6). Within all other months sampled, mean microplastic abundance was comparable between Kau Point, Oriental Bay, and Scorching Bay (Figure 3.6).

Table 3.3: Results of a pairwise comparison, examining between-site variation (Kau Point, Oriental Bay, and Scorching Bay) of microplastic abundance within *M. galloprovincialis* samples, with \* indicating significant differences at  $p = 0.05$ .

Month	Site	Estimate	SE	Z.ratio	P.value	
Feb19	KP - OB	-0.00717	0.120	-0.060	0.9980	
	KP - SCB	-0.25848	0.113	-2.289	0.0575	
	OB - SCB	-0.25131	0.113	-2.230	0.0662	
May19	KP - OB	-0.42286	0.295	-1.433	0.3241	
	KP - SCB	-0.35140	0.299	-1.173	0.4690	
	OB - SCB	0.07146	0.267	0.267	0.9614	
Aug19	KP - OB	-0.62415	0.226	-2.759	0.0160	*
	KP - SCB	-0.20972	0.246	-0.854	0.6696	
	OB - SCB	0.41443	0.212	1.956	0.1233	
Nov19	KP - OB	-0.19237	0.235	-0.818	0.6918	
	KP - SCB	0.31845	0.268	1.187	0.4609	
	OB - SCB	0.51083	0.258	1.978	0.1175	
Feb20	KP - OB	0.18232	0.214	0.852	0.6708	
	KP - SCB	0.28768	0.220	1.305	0.3925	
	OB - SCB	0.10536	0.230	0.459	0.8906	

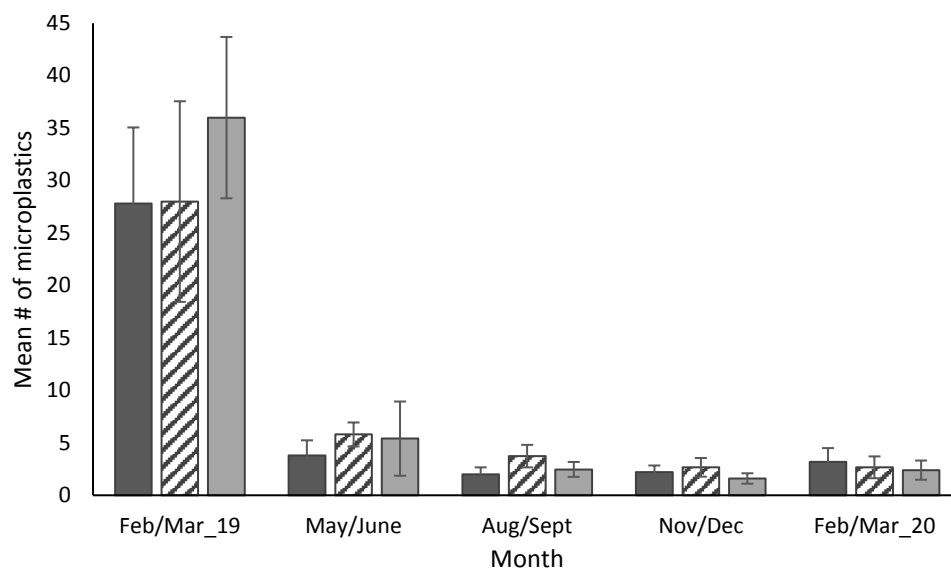


Figure 3.6: Average (+/- 95% confidence interval) microplastic abundances per mussel between sites within each collection month, where black bars represent Kau Point, striped bars represent Oriental Bay, and grey bars represent Scorching Bay.

### Beach sediment

#### Monthly variation

Similar to the mean microplastic abundance within *M. galloprovincialis* tissues, the pollutant load in beach sediment cores (each sample ~ 400 cm<sup>3</sup>) was also highly variable. However, average microplastic load peaked in May 2019, as opposed to February 2019 (Figure 3.7). Sediments collected in May 2019 were up to 11 times greater than the smallest monthly average, in November 2019 (Figure 3.7). A pairwise comparison between months indicated that all sampled months were significantly different than each other (Table 3.4).

Table 3.4: Results of between-month variation (February 2019, May 2019, August 2019, November 2019, and February 2020) on microplastic abundance within pooled beach sediment samples, where \* indicates significant differences at  $p = 0.05$ .

Month	Estimate	SE	Z.ratio	P.value	
Aug19 - Feb19	-0.347	0.0520	-6.669	<0.0001	*
Aug19 - Feb20	0.333	0.0455	7.320	<0.0001	*
Aug19 - May19	-1.448	0.0392	-36.941	<0.0001	*
Aug19 - Nov19	0.951	0.0555	17.130	<0.0001	*
Feb19 - Feb20	0.680	0.0554	12.276	<0.0001	*
Feb19 - May19	-1.101	0.0503	-21.876	<0.0001	*
Feb19 - Nov19	1.297	0.0638	20.323	<0.0001	*
Feb20 - May19	-1.781	0.0436	-40.860	<0.0001	*
Feb20 - Nov19	0.617	0.0587	10.519	<0.0001	*
May19 - Nov19	2.399	0.0539	44.494	<0.0001	*

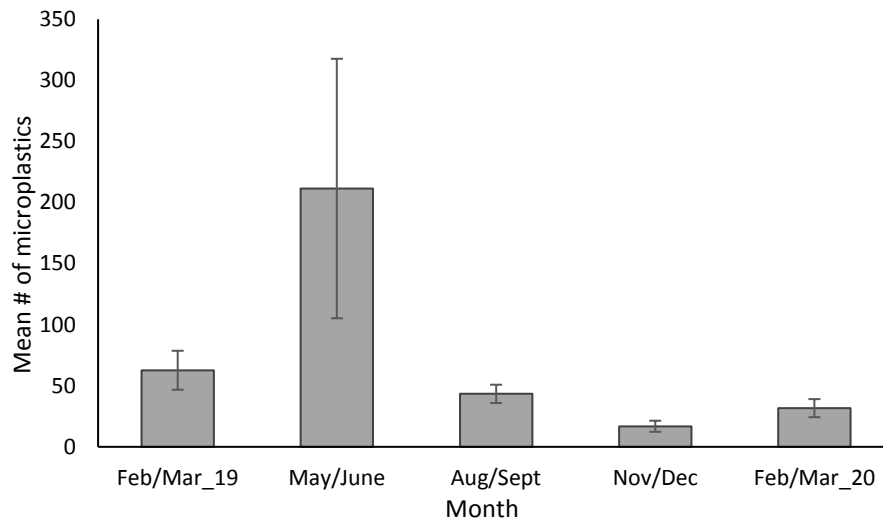


Figure 3.7: Mean (+/- 95% confidence interval) abundance of microplastics observed within beach sediment samples of approximately 400 cm<sup>3</sup>, collected in February 2019, May 2019, August 2019, November 2019, and February 2020.

### Weekly variation

Average microplastic abundance in beach sediment cores (each sample ~ 400 cm<sup>3</sup>) was slightly more variable on a weekly basis than in *M. galloprovincialis* tissues. There were no statistically significant differences between weeks in August 2019 (Table 3.5), however week 1 in November 2019 had a higher amount of microplastics on average compared to week 2 (Table 3.5). In addition, week 1 of February 2020 had a higher number of microplastics contained within the sediments than both subsequent weeks of the same month. (Figure 3.8).

Table 3.5: Results of between-week variation (Weeks 1, 2, and 3 respectively) on microplastic abundance within beach sediment samples of approximately 400 cm<sup>3</sup>, collected in August 2019, November 2019, and February 2020. \* indicates significant differences at  $p = 0.05$ .

Month	Collection	Estimate	SE	Z.ratio	P.value	
Aug19	A - B	-0.0126	0.0710	-0.178	0.9828	
	A - C	0.0441	0.0720	0.612	0.8135	
	B - C	0.0567	0.0718	0.79	0.7094	
Nov19	A - B	0.2896	0.1169	2.487	0.0353	*
	A - C	0.0918	0.1107	0.829	0.6849	
	B - C	-0.1978	0.1193	-1.659	0.2212	
Feb20	A - B	0.7975	0.0852	9.365	<0.0001	*
	A - C	0.7441	0.0836	8.897	<0.0001	*
	B - C	-0.0535	0.0987	-0.543	0.8503	

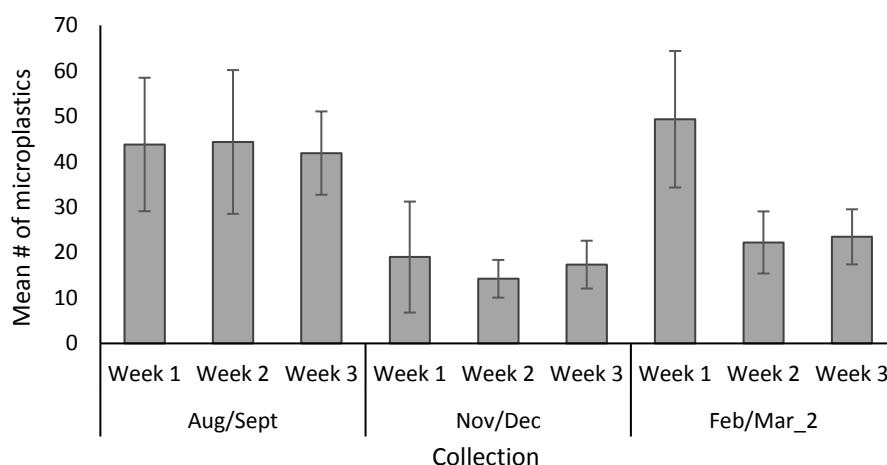


Figure 3.8: Average (+/- 95% confidence interval) abundance of microplastic particles within beach sediment samples of approximately 400 cm<sup>3</sup>, with variation observed between weekly collections in Aug/Sept '19, Nov/Dec '19, and Feb/Mar '20.

### Spatial variation

In contrast to *M. galloprovincialis* samples, microplastic abundance within beach sediments appears to be more spatially variable within months, with site variability observed in three out of five months. In February 2019, microplastic abundance was significantly different at Oriental Bay, while in May 2019 each site was dissimilar in microplastic load from the others (Table 3.6), with Seatoun having the greatest difference. Values in Seatoun during May 2019 were up to two times greater than average microplastic abundance in Balaena Bay or Oriental Bay (Figure 3.9). Thirdly, in February 2020, Balaena Bay differed from both other sites (Table 3.6), with less microplastics on average in its sediments than the other two sites (Figure 3.9). Consequently, the sites where difference in microplastic abundance was observed was not ubiquitous across all sampled months.

Table 3.6: Results of between-site variation (Balaena Bay, Oriental Bay, and Scorching Bay) on microplastic abundance within beach sediment samples of approximately 400 cm<sup>3</sup> across all collection months. \* indicates significant differences at p = 0.05.

Month	Site	Estimate	SE	Z.ratio	P.value	
Feb19	BB - OB	-0.2877	0.0986	-2.918	0.0099	*
	BB - ST	0.2231	0.1118	1.996	0.1132	
	OB - ST	0.5108	0.1054	4.846	<0.0001	*
May19	BB - OB	0.4316	0.0724	5.964	<0.0001	*
	BB - ST	-0.8262	0.0544	-15.174	<0.0001	*
	OB - ST	-1.2577	0.0639	-19.698	<0.0001	*
Aug19	BB - OB	-0.0153	0.0715	-0.215	0.9749	
	BB - ST	0.0000	0.0718	0.000	1.0000	
	OB - ST	0.0153	0.0715	0.215	0.9749	
Nov19	BB - OB	-0.1881	0.1123	-1.675	0.2150	
	BB - ST	0.0715	0.1196	0.597	0.8215	
	OB - ST	0.2595	0.1145	2.265	0.0608	
Feb20	BB - OB	-0.4580	0.0882	-5.195	<0.0001	*
	BB - ST	-0.3991	0.0892	-4.474	<0.0001	*
	OB - ST	0.0589	0.0788	0.748	0.7348	

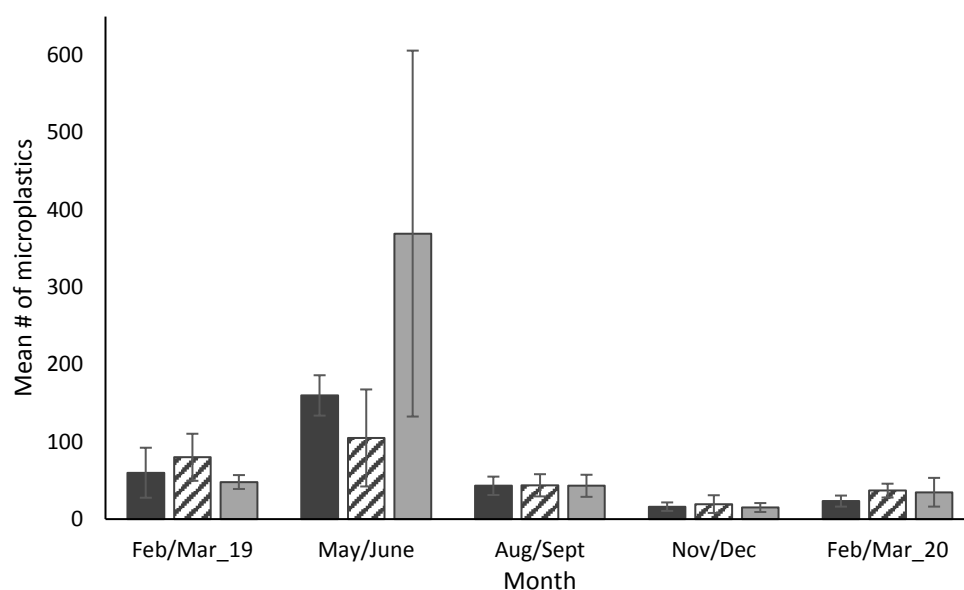


Figure 3.9: Average (+/- 95% confidence interval) microplastic abundances between sites within each sediment collection month, where black bars represent sediment cores (approximately 400 cm<sup>3</sup>) collected in Balaena Bay, striped bars represent Oriental Bay, and grey bars represent Seatoun.

### *Surface water tows*

Microplastic abundance between the months of March – November 2019 appeared to be dynamic (Figure 3.10), with the highest number of synthetic particles within a single 200 m long neuston net transect recorded in March 2019, at Matiu-Somes Island (n = 107). The lowest number of particles collected in a single tow occurred during September 2019 – also at

Matiu-Somes Island ( $n = 19$ ). Despite the apparent differences between the sample months, a Kruskal-Wallis test suggests that there is no significant difference in microplastic abundance between different months ( $p = 0.129$ ). Spatially, Figure 3.10 suggests there may be similarities between Evans Bay and Matiu/Somes Island, as the tows produced comparable amounts of microplastic particles within each month that was sampled. These results must be interpreted with caution, however, due to the small sample size of water surface tows able to be collected during the study.

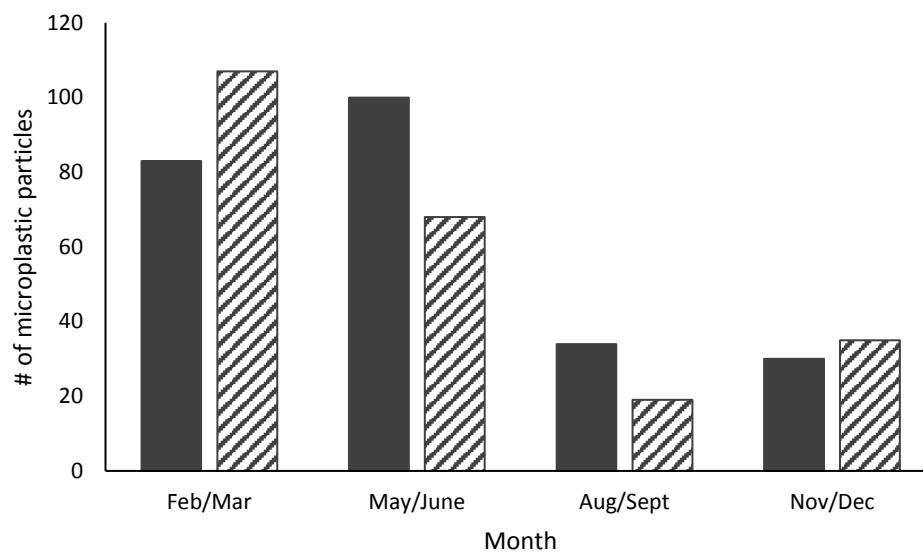


Figure 3.10: Total microplastic abundance per 200 m neuston net transect between collection sites, at each 3-monthly sampling tow, where black bars represent Evans Bay and striped bars represent Matiu-Somes Island.

### *Plastic characteristics*

#### Type of particle

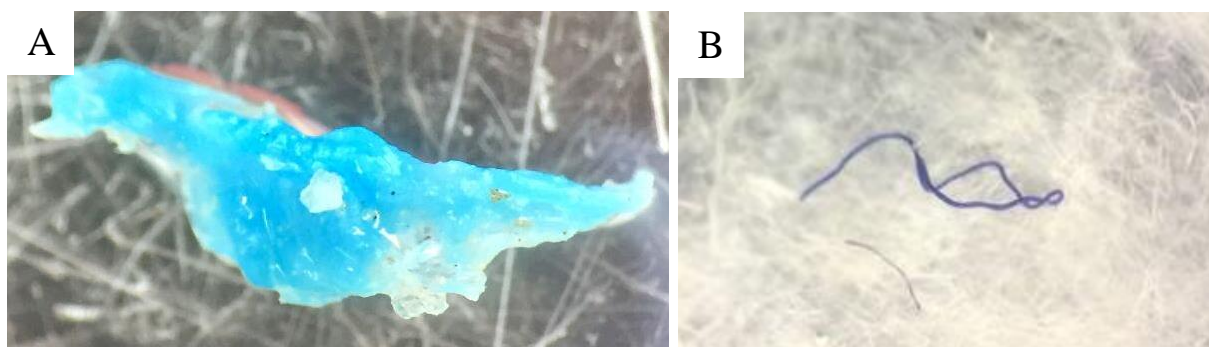


Figure 3.11: Two examples of synthetic particles collected within this study, where A) was a large blue fragment found in a *M. galloprovincialis* individual, whilst B) shows two fibres (black and blue) of medium and small sizes respectively, found within sea surface samples.



Multiple types of inorganic particles were found in all three sources of collection for this study, including those shown in Figure 3.11. Synthetic fibres were the predominant plastic type throughout all three sources, accounting for 90.5% of all synthetic particles within sediments collected along the high tide line, 94.2% in surface water tows, and 95.8% within *M. galloprovincialis* tissue. Plastic fragments were the second-most abundant particle type in all three sources, although these accounting for a mere 3.6% of total inorganic particles in *M. galloprovincialis*, 5.2% of surface water samples, and 9.2% of particles collected within sediments. All other occurrences of various inorganic particles within *M. galloprovincialis*, sediment, and surface water tows are negligible (Table 3.7), with synthetic fibres and plastic fragments the only consistent types of microplastic found throughout the study (Figure 3.12). The difference in percentage of each particle type was significant in the three sources ( $\chi^2 (4) = 38.939$ ,  $p = <0.0001$ ), particularly as microfibres comprised the highest percentage in *M. galloprovincialis* samples, while microplastic fragments were more common in beach sediment samples as opposed to the other two sources (Figure 3.12).

Table 3.7: Count data investigating total number of synthetic particles within *M. galloprovincialis* tissues, sediment, and water tows collected during the scope of this study.

	Bead	Fabric	Fibre	Foil	Fragment	Macro	Virgin pellet	Paint
Mussel	1	0	845	1	32	3	0	0
Sediment	0	0	4495	0	458	12	3	1
Water	0	1	451	0	25	2	0	0

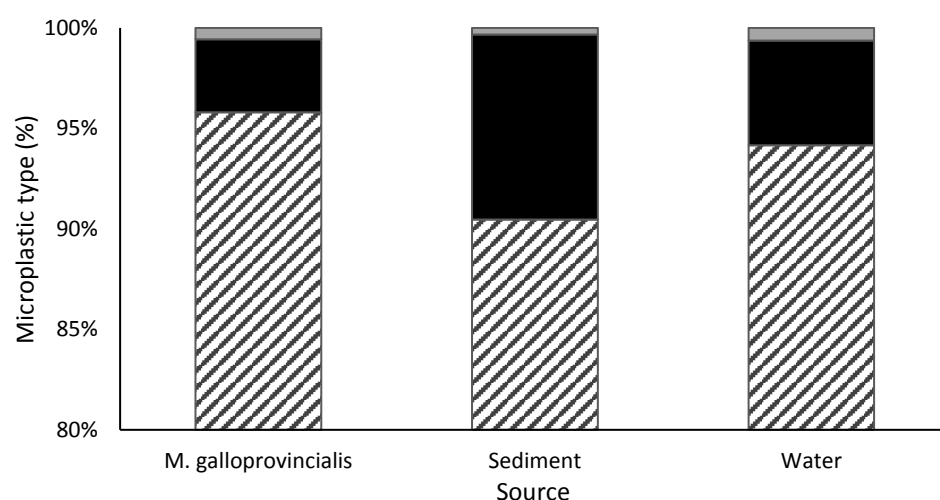


Figure 3.12: Percentages of the total count data between February 2019 and February 2020, for all different plastic type. Data is pooled across sources. Striped bars represent the percentage of microfibres, black bars represent the percentage of fragments, and open bars represent the percentage of all other plastic types – beads, fabric, foil, macroplastic, virgin plastic pellets, and paint chips.

### Size of plastic particles

A total of 6,312 synthetic particles were detected within the samples collected for this study. Of these, medium-sized microplastics (1-2 mm) were most common throughout all three sources; *M. galloprovincialis* tissues, beach sediments, and surface water tows (Table 3.8). This size category comprised between 40 – 44% of all synthetic particles observed, while small and large particles were more widely varied between the three sources (Figure 3.13). The difference in the proportions was shown to be significant between size classes and the three sources ( $\chi^2 (4) = 51.828$ ,  $p = <0.0001$ ).

Table 3.8: Count data investigating size distributions of synthetic particles within *M. galloprovincialis* tissues, sediment, and water tows collected during the scope of this study. Count data not inclusive of macroplastic particles >5 mm in length or diameter.

	Small	Medium	Large
Mussel	208	355	316
Sediment	1501	2215	1240
Water	128	201	148

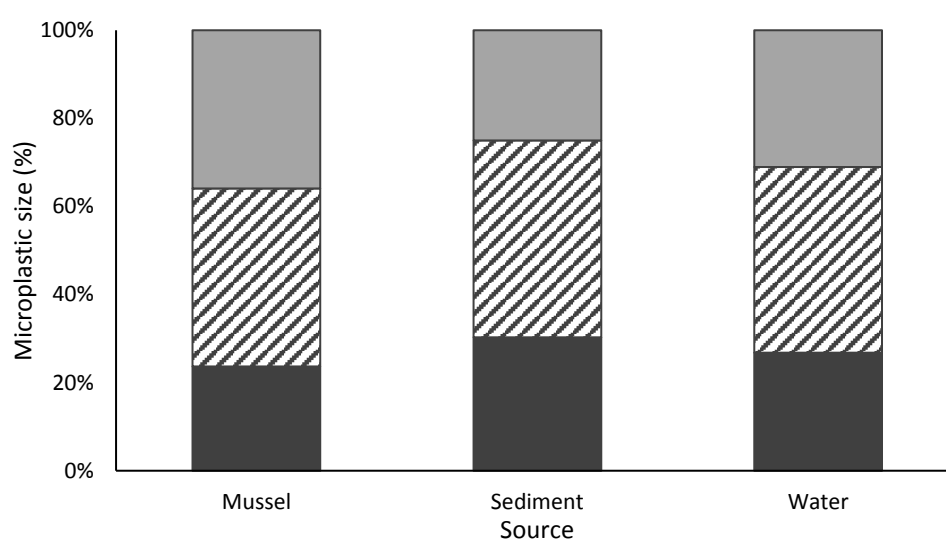


Figure 3.13: Microplastic size distributions within *M. galloprovincialis*, sediment, and surface water tows, expressed as a percentage. Black bars represent the percentage of small particles (< 1 mm), striped bars represent medium (1 – 2 mm), and grey bars represent large particles (> 2 – 5 mm).

### Colour of particles

The colours of synthetic particles were found to range significantly throughout *M. galloprovincialis*, beach sediment, and water surface samples. Black and blue microplastics were the most prevalent varieties, accounting for between 25 – 52% of each source. All other colours found within the samples were far less predominant, as demonstrated in Figure 3.14. The difference in percentage of each particle colour (black, blue, colourless, and other) was significant in the three sources ( $\chi^2 (6) = 294.52$ ,  $p = <0.0001$ ), supporting the count data

shown in Table 3.9. Black particles were particularly of highest prevalence in the sediment and water samples, while blue particles comprised a far greater proportion of the colours found within *M. galloprovincialis* samples.

Table 3.9: Count data investigating colour distributions of synthetic particles found within *M. galloprovincialis* tissues, sediment, and water tows collected during the scope of this study.

	Black	Blue	Colourless	Green	Orange	Purple	Red	White	Yellow
Mussel	229	461	126	10	10	2	41	3	1
Sediment	2350	1981	183	61	39	25	145	149	36
Water	229	130	37	13	13	5	33	11	8

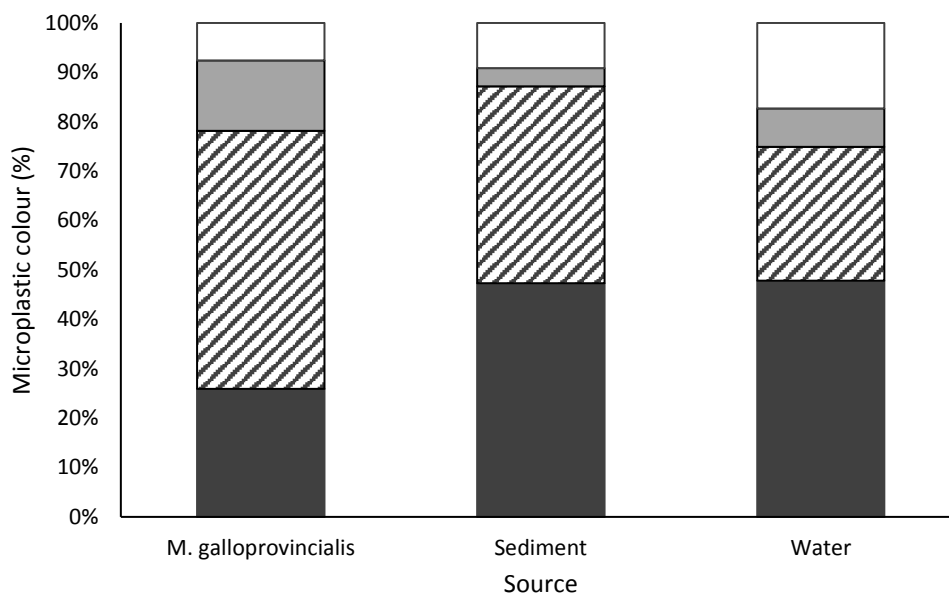


Figure 3.14: Microplastic colour distributions within *M. galloprovincialis*, sediment, and surface water tows, expressed as stacked percentages of the total count data for each source. Black bars represent the percentage of black particles, striped bars represent blue particles, grey bars represent colourless particles, and open bars represent the total percentage of all other colours (green, orange, pink, purple, red, white, and yellow).

## Discussion

This study illustrated that microplastic abundance within Wellington Harbour is highly dynamic on a variety of spatial and temporal scales, both within and among the three different sources examined: *M. galloprovincialis* tissues, beach sediments, and surface water tows. As hypothesized, the abundance and distribution of microplastic pollution was reasonably variable between the months and sites sampled, however less so between weeks. Furthermore, tissues of *M. galloprovincialis* individuals had a low abundance of microplastic particles on average, as hypothesized, although the pollutant load within all three sources were relatively on par with what has been found elsewhere in the world's coastal environments. This difference between sources is likely due to each mussel filtering a relatively small volume of seawater, in addition to their ability to egest synthetic particles within a relatively short period following ingestion (Woods *et al.*, 2018; Gonçalves *et al.*, 2019). The peak microplastic load for *M. galloprovincialis* tissues and surface water tows was observed in the samples collected during February/March, however for beach sediments the highest level of plastic pollution was observed in May/June. This may indicate that microplastics in Wellington Harbour do not follow the same temporal trend across all sources, or that different environmental factors impact the various habitats and organisms in separate ways.

Spatial differences were not as pronounced across the board as the temporal variation that occurred. This was particularly true for the *M. galloprovincialis* tissues, as Scorching Bay samples were similar to the other sites over all months. Oriental Bay individuals contained more synthetic particles than those collected from Kau Point in August 2019, but this was the only instance of significant variation. Interestingly, beach sediments experienced much higher levels of spatial variation, occurring over all three sites. This may also be due to environmental drivers that affect loading of plastic in beach sediment, however human interaction with beach surfaces may also play a role (Barnes *et al.*, 2009; Agamuthu *et al.*, 2019).

As hypothesized, microfibrils were the most common type of synthetic particle detected in all three sources. Furthermore, the colour and size of particles ranged significantly throughout mussel tissue, beach sediment, and water surface samples. Black and blue particles were highly abundant in all three sources, whilst overall, size was the particle characteristic that was most evenly distributed, with particles measuring between 1 – 2 mm in length found to be the most prevalent size class observed in all three sources.

### *Microplastic abundance & distribution in Mytilus galloprovincialis*

Mussels are ecologically significant in coastal marine ecosystems, and an integral role in benthic-pelagic coupling as a primary consumer that directly interacts with the surrounding environment. Consequently, it has been suggested that they are valuable bioindicators of microplastic pollution (Beyer *et al.*, 2017; Brate *et al.*, 2018; Li *et al.*, 2019; Scott *et al.*, 2019), thus helping to demonstrate the rate at which microplastics can enter the food web.

In this multi-pronged survey of Wellington Harbour, it is significant that synthetic particles were found within the tissues of *M. galloprovincialis*, which has not been documented in the Southern Hemisphere until now. This suggests that the uptake of microplastic pollutants can occur through either ingestion or adherence to their tissues (Kolandasamy *et al.*, 2018; Webb *et al.*, 2019), but the majority of particles are egested as opposed to accumulating in the organism's internal tissues (Capolupo *et al.*, 2018; Gonçalves *et al.*, 2019).

Some laboratory studies have begun to look at the accumulation vs. egestion of pollutants such as microplastics and heavy metals, within various mussel species. Estimates vary regarding how long microplastics are able to persist in mussels, but one such study reports that particles translocated from the digestive system to the circulatory system of *Mytilus edulis* and remained there for over 48 days (Browne *et al.*, 2008). In contrast, a different study reported that after introducing 30 microfibrils mL<sup>-1</sup> to *M. edulis* mussels, approximately 71% of these were quickly released as pseudofeces, and 9% ingested after a period of 3 hours (Woods *et al.*, 2018). For *M. galloprovincialis* mussels, laboratory studies have focused upon microplastics such as polystyrene up until this point, with one paper providing evidence for rapid processing and egestion of the particles (Gonçalves *et al.*, 2019). However, microplastics were still retained within the digestive system for 7 days after ingestion (Gonçalves *et al.*, 2019). On the other hand, a larval study demonstrated that polystyrene microplastics were retained for up to 8 days in the digestive tract after ingestion (Capolupo *et al.*, 2018). Both studies provided evidence for microplastics remaining in the digestive tract only, rather than being translocated to the gills or other internal tissues; indicating that microplastics are preferentially taken up when feeding (Rivera-Hernández *et al.*, 2019). Since *M. galloprovincialis* mussels were digested whole during this study, an individual analysis of where the microplastic particles were located was unable to occur; in future studies this would provide evidence for short-term versus long-term retainment.

Interestingly, spatial trends indicated that microplastic load within *M. galloprovincialis* mussels around different Wellington Harbour sites that are on average 1.3 km apart (straightline distance) do not vary much. These results indicate that variation across the scale of sites separated by a few kilometres is less important than temporal variation in contributing to patterns of microplastic abundance in *M. galloprovincialis* mussels in Wellington Harbour. This result is similar to the spatial patterns discussed in Webb *et al.* (2019), where microplastic abundance within *P. canaliculus* samples collected from around New Zealand (including the Auckland, Wellington, and Canterbury regions) was not found to be significantly associated with location. Although this study looked at finer spatial trends in comparison to Webb *et al.* (2019), it can still be suggested that the same principles apply. As a semi-enclosed harbour, Wellington Harbour experiences high levels of urbanisation, however the intensity of human activity is likely to be more constant than other coastal areas in the Northern Hemisphere that have reported significant spatial trends of microplastic abundance for various mussel species. For example, Li *et al.* (2016) conducted a study across approximately 2/3 of the total mainland coastline China, looking at the microplastic pollution load within the *Mytilus edulis* population. Their results suggested that the microplastic load was significantly higher in areas that undergo intensive human activities, and less so in the more remote areas. These differences could be – in part – due to differences in sampling methodologies, but it is also feasible that there may be higher levels of variability in pollutant loads across regions with intensive human activity.

Apart from the mussel samples collected in February 2019, there was generally a low abundance of microplastic particles contained within *M. galloprovincialis* tissues. The microplastic load ranged between 0 – 11 (May 2019 – February 2020 samples), with an average of 2.8 particles per individual or  $0.30 \pm 0.04$  particles  $\text{g}^{-1}$  (wet weight). These results are within a similar range to other mussel studies, both within New Zealand and globally. In New Zealand, Webb *et al.* (2019) reported 0 – 1.5 particles per *Perna canaliculus* mussel, while for the *M. edulis* spp. some reports suggest a microplastic load in the range of  $0.36 \pm 0.07$  particles  $\text{g}^{-1}$  from a mussel farm in Germany (Van Cauwenberghe & Janssen, 2014) or  $0.3 \pm 0.2$  particles  $\text{g}^{-1}$  on the French/Belgian/Dutch coastline (Van Cauwenberghe *et al.*, 2015). For *M. galloprovincialis* mussels, one study reported that this species may contain up to  $2.33 \pm 0.2$  particles  $\text{g}^{-1}$  for individuals along the South African coastline (Sparks, 2020), while a study on coast of the Adriatic Sea reported 1.06 – 1.33 fragments  $\text{g}^{-1}$  and 0.62 – 0.63 fibres  $\text{g}^{-1}$  (Gomiero *et al.*, 2019). In saying this, however, many variables are at play when

investigating microplastic abundance in bivalves and other taxa. Geographic region is likely of high importance, as some coastal areas may be more heavily influenced by urbanisation, or subject to more pronounced environmental drivers. Furthermore, as there is no standard laboratory protocol for assessing microplastic load in mussel species, many different digestion methods are used, which may yield varying results. Until recently, for example, the use of Nitric acid was popular for digestion of soft tissue, however it has since been reported that this method results in degradation of some synthetic particles (Claessens *et al.*, 2013; Dehaut *et al.*, 2016; Karami *et al.*, 2017). Regardless, these results are generally in line with those found in other published studies, suggesting that the maximum processing rates of microplastic particles are similar throughout the world and across multiple mussel species.

#### *Microplastic abundance & distribution in beach sediments*

This thesis has provided the first effort to quantify microplastic loads in Wellington beach sediments in the 21<sup>st</sup> century. The substantial quantity of particles found in this study is likely due to a combination of factors, but environmental drivers are thought to be highly influential. Wave stress and regular high tides, for example, are generally considered to be integral factors, as they push the particles onto the shore, resulting in a high concentration (Young & Elliott, 2016). Using sediment cores with an approximate volume of 400cm<sup>3</sup> per sample, the extrapolated average abundance of microplastics within sandy beach sediments was  $124.9 \pm 35.7$  particles kg<sup>-1</sup> (wet weight). This estimation is on the lower end of global beach sediment microplastic approximations in the Northern Hemisphere, but surprisingly appears to be higher than the abundances reported in other Southern Hemisphere studies – including those carried out in other regions of New Zealand. Clunies-Ross *et al.* (2016) reported 0 – 45.4 particles kg<sup>-1</sup> within the Canterbury region, while Bridson *et al.* (2020) only reported an average of 6 particles kg<sup>-1</sup> in the Auckland region. In comparison, a study in Belgium reported 53 – 390 particles kg<sup>-1</sup> (Claessons *et al.*, 2011), therefore bearing more similarities to the results from this study.

The large abundance in Wellington Harbour may possibly indicate that there are higher levels of microplastic pollution within the region's marine environment. In saying that, however, at this stage it cannot yet be determined whether the microplastic particles examined during this study stem from a local origin such as the Hutt River and urban inputs around the region, or whether they come from further afield – flowing into the harbour via the Cook Strait entrance. 40 years ago, Gregory (1978) demonstrated that one microplastic type, virgin plastic pellets, were of the highest abundance around locales with large populations – like

Wellington – indicating that the plastics may have local origins. More recently, microplastics were found in urban streams around New Zealand, including from the Hutt River, therefore supporting the idea that at least some plastic inputs occur upstream (Mora-Teddy & Matthaei, 2020). Bridson *et al.* (2020) however, suggests that a significant proportion of the microplastic load found around New Zealand may originate from nations such as Australia and the South-east of Asia. This is particularly likely when considering the major oceanic circulation systems in the Southern Hemisphere, where the New Zealand archipelago encounters the East Australian current (Bridson *et al.*, 2020), as well as the report from Eriksen *et al.* (2014), where New Zealand is included in an accumulation zone for floating plastic litter. Regardless of whether microplastics found in Wellington Harbour originate locally or from afar, it is still possible that once they are in the harbour, they are retained in the region for a significant period.

Wellington Harbour is considered a well-mixed system (Maxwell, 1956; Gilmour, 1960; Booth, 1975), with most local discrepancies quickly dispersed throughout the 76 km<sup>2</sup> area. However, Booth (1975) also reported that the harbour is relatively cut off from the Cook Strait, with much of the mixing occurring within the constraints of the Harbour. This does suggest that it would be possible for microplastics to have a long residence time inside the Harbour's system, with the possibility of also collecting within the semi-enclosed region. Consequently, if pollutant loads are significant with the additional ability to collect over time, it is probable that microplastic sinks such as beaches are a common deposition point for the particles. On the other hand, environmental variables further complicate the issue, in that beaches may not be permanent sinks. Moore (2008) reports that onshore winds will often push floating debris onto shorelines, whilst offshore winds will have the opposite effect. Furthermore, a lack of wind promotes the accumulation of synthetic particles on the coastline as they have time to settle into the sediment, whilst strong winds often displace the plastics – possibly resuspending the particles back into the water column (Oigman-Pszczol & Creed, 2007; Costa *et al.*, 2011; Agamuthu *et al.*, 2019). These variables make it extremely difficult to accurately predict the spatial and temporal trends of microplastic abundance and distribution, even in semi-enclosed environments such as Wellington Harbour.

There was a higher degree of spatial variation in microplastic abundance for beaches than mussels, despite the similar, relatively close distances between sites (approximately 1.5 km – straightline distance). Differing levels of human activity throughout the year has often been attributed to this changeable load in various studies (Bridson *et al.*, 2020), as movement of



particles on the top layers of beach sediment often results in smaller items being dispersed or buried (Agamuthu *et al.*, 2019). However, other contributing factors to this variation are likely to be differences amongst rates of sediment erosion, the variable topography of the beaches (Ribic *et al.*, 2012; Agamuthu *et al.*, 2019), and the direction that the beach faces in relation to the prevailing Wellington winds. In this study, the approximate directions of each beach differ, with Oriental Bay beach facing North, Seatoun beach facing North-East, and Balaena Bay facing South-East. As stated in Oigman-Pszczol & Creed (2007), Costa *et al.* (2011), and Agamuthu *et al.* (2019), beaches that are not as readily exposed to prevailing winds often accumulate microplastics to a greater degree, as the lack of wind force enables the pollutants to settle on the coastline. Therefore, the differing coastline directions, in conjunction with some or all of the other social and environmental factors, may contribute to the irregular spatial variation observed during this study.

#### *Microplastic abundance & distribution in surface water tows*

Temporal trends in microplastic abundance peaked at similar periods throughout the three sources sampled. Tissues of *M. galloprovincialis* mussels had the highest load in February 2019, also observed in water surface tows. Beach sediments, however, had a slight delay, with their peak microplastic load occurring in the next sampling period, May 2019.

Interestingly, the temporal trends seen in mussel tissues and water samples followed a similar curve to each other, while beach sediment data experienced more drastic peaks and troughs. This is possibly reminiscent of the results found in Qu *et al.* (2018), who report a correlation between the abundance of microplastics in surface waters and within mussels, but no correlation to the pollutant load in beach sediments within the same areas. Furthermore, Qu *et al.* (2018) suggest that the microplastic abundances present within mussel tissues are dependent upon the quantities in the water column. This determination cannot be made in this study, but the possibility of a correlation is intriguing, nonetheless.

This is the first study in New Zealand that has surveyed surface waters for microplastic occurrence, although unfortunately limited in scope due to logistical constraints. Worldwide, particularly within the Northern Hemisphere, surface waters have been studied extensively, providing valuable estimations of micro- and macroplastic pollution. Therefore, although the results from this study in Wellington Harbour should be interpreted with caution, it provides a baseline for future surface water studies investigating abundance and distribution of plastic in New Zealand coastal waters. These transects collected between 15 – 107 particles per 200 m, with an average of  $59.5 \pm 23.8$  particles per 200 m. Global results from sea surface tows

are extremely variable, with one study reporting a maximum of 157,000 particles km<sup>-2</sup> in the North-East Atlantic (Maes *et al.*, 2017), while studies carried out by the North-Pacific gyre have previously recorded up to 300,000 particles km<sup>-2</sup> (Moore *et al.*, 2001). However, methodologies for assessing differences and similarities in global microplastic abundance are still varied in almost every aspect (Galgani *et al.*, 2015). With no standard methodologies across the board, there are limitations upon creating a comprehensive understanding of marine plastic pollution, let alone taking steps to manage and reduce it. Furthermore, microplastic abundance and distribution within surface waters are again likely to be heavily impacted by environmental drivers such as vertical mixing due to wind strength, resulting in even larger variance between scientific studies.

### *Conclusion*

This study is the first of its kind in New Zealand, and one of the first globally. Taking a multi-pronged approach to assessing microplastic pollution in a localised area provides a valuable baseline for further research. Surface waters, beach sediments, and filter-feeding bivalves such as *Mytilus galloprovincialis* have all been studied comprehensively in other nations, particularly throughout the Northern Hemisphere. Therefore, through investigating possible temporal and spatial patterns of microplastic abundance and distribution, future studies may build upon the initial trends observed.

## 4. General discussion

Understanding how the abundance and distribution of microplastic debris varies in the marine environment is integral to coming up with mitigation, management, and prevention strategies. However, unless there is a global understanding of where this pollutant is, and at what concentrations, we cannot begin to effectively reduce the impacts upon affected marine species. Plastic pollution has been an ever-increasing issue since mass production began in the 1940's (Cole *et al.*, 2011), particularly as its impacts were initially underestimated (Colton *et al.*, 1974; Zitko & Hanlon, 1991; Gregory, 1996). In the marine environment, the characteristics of plastic enable it to persist for an extended period of time and penetrate numerous ecosystems including the sea surface, the benthos & deep sea, coastal sediments, polar environments, and more (e.g. Van Cauwenberghe *et al.*, 2013; Obbard *et al.*, 2014; Woodall *et al.*, 2014; Cózar *et al.*, 2015). However, most of this current information has arisen from studies carried out in the Northern Hemisphere, since it is more heavily populated and thus has a greater number of research facilities investigating plastic pollution in the oceans (Lusher, 2015; Avio *et al.*, 2017). Consequently, until recently it has been relatively unknown as to whether oceanic currents have circulated microplastics throughout the world, or alternatively whether direct and indirect inputs of plastic pollution are as significant in the Southern Hemisphere as they are in the Northern Hemisphere.

Variation in microplastic abundance can impact the associated marine biota. If concentration of microplastic debris is high, this interaction is likely to be significant, therefore increasing the associated risk for negative effects on individuals (Agamuthu *et al.*, 2019). Direct interactions such as ingestion or adherence to tissues are typically observed in taxa that are filter-feeders or suspension-feeders, thus occupying the lower trophic levels. However, once plastics have entered the food web, there is evidence to suggest that the particles – and the chemicals associated with them – can be transported up throughout the food web and bioaccumulate within internal tissues (Kelly *et al.*, 2007). Such interactions have been reported to have negative impacts upon the physiology and behaviour of organisms (Andrady, 2011; Wright, 2013; Gall & Thompson, 2015; Agamuthu *et al.*, 2019) and also possibly human health (Van Cauwenberghe & Janssen, 2014; Agamuthu *et al.*, 2019) – although this facet of research is still in its infancy (Vandermeersch *et al.*, 2015).

In this study, I found that microplastics are found in many habitats throughout Wellington Harbour, as well as in the tissues of intertidal *M. galloprovincialis* mussels. The

concentration within *M. galloprovincialis* specimens was comparable to the microplastic loads reported within other studies that have examined mussel species, including one that was carried out in New Zealand on *P. canaliculus* (Webb *et al.*, 2019). Microplastic concentrations within beach sediments and water surface tows were also in a similar range to what other studies have reported, however on a global scale there is an extremely broad range of estimates for the concentration of microplastics in these habitats. This discrepancy is thought to be a result of the wide range of environmental variables that drive the movement of plastics within the water column and onto the beach, including wind and wave exposure, sediment erosion, and direction of the coastline, along with the inconsistency between different sampling methodologies used in various studies.

Sampling methodologies differ widely in almost every way across the breadth of current microplastic research, from field collections to laboratory analyses. For surface water sampling, this variation often arises from the type of net or collection technique used, whether that be a neuston net, Continuous Plankton Recorder, manta trawl, the ‘grab’ technique, or a saltwater intake pump (Figure 4.1) (Robinson *et al.*, 2014; Setälä *et al.*, 2016; Barrows *et al.*, 2017; Cincinelli *et al.*, 2017; Green *et al.*, 2018). These all have different mesh sizes and varying success in capturing environmental microplastics. The differences are exacerbated in the laboratory setting, not only for surface water samples, but those from sediments and organisms also. The extraction of synthetic particles from different sources can be done in many ways including density flotations, acidic separations, and the digestion of soft tissue using numerous kinds of acids, alkali, and oxidation chemicals, all of which is further demonstrated by Miller *et al.* (2017) who has compiled a review of the methodologies used in a range of studies. This lack of consistency across the literature not only makes it difficult to know which methods to follow, but also increases the likelihood of sampling error when assessing pollutant load, simultaneously hindering the chance to make progress at all scales within the field.

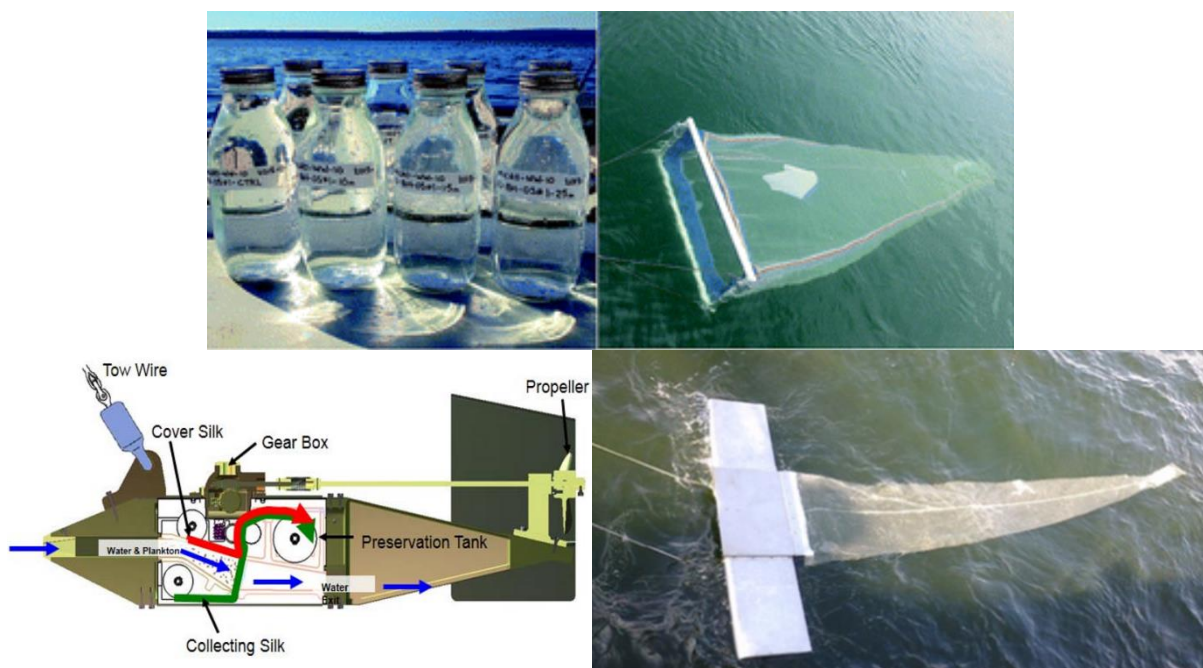


Figure 4.1: Examples of the various sea surface sampling methods that are commonly used for assessing microplastic load. A) is a ‘grab’ system, B) a neuston net, C) a Continuous Plankton Recorder (CPR), and D) is a manta trawl. Image credit: A) & B) Barrows *et al.*, 2017; C) Robinson *et al.*, and D) Setälä *et al.*, 2016.

In general, the abundance and distribution of microplastic particles was highly dynamic at both the relatively small scale within Wellington Harbour, and in the large scale of the surface waters between New Zealand and the Ross Sea. This high level of variability is one of the main conclusions to come from this research and is indicative of the complex processes that dictate the movement of plastic pollutants. Plastic materials of all size classes are renowned for being lightweight yet durable, which subsequently makes it difficult to predict and model where they are likely to move or end up in the world’s oceans (Andrady, 2011). In saying that, this study provides preliminary evidence that both fine-scale (within an urbanised harbour) and wide-scale (across multiple oceanographic zones) spatial differences are not as pronounced as temporal differences. Spatial trends have been studied in far more depth, due to macropatterns such as the trend for plastic to accumulate in gyres and along frontal systems (Moore *et al.*, 2001; Van Sebille *et al.*, 2012; Fazey & Ryan, 2016). There have only been a limited amount of studies that examine both spatial and temporal trends in microplastic abundance and distribution, however of those that do, both space and time are thought to be useful predictors to model current and future abundance of this marine pollutant (Isobe *et al.*, 2019).

In accordance to predictions, microfibrils were the most prevalent morphotype of microplastic found throughout both Wellington Harbour and the transects between New Zealand and the Ross Sea. These results were ubiquitous across sea surface tows, beach sediment surveys, and

contamination within *M. galloprovincialis* mussels. This is consistent with reports of global microplastic estimates, some of which predict between 90 - 95% of all marine microplastic debris to be in the form of microfibrils (Galgani *et al.*, 2015; Cesa *et al.*, 2017; Barrows *et al.*, 2017; Woods *et al.*, 2018). As opposed to other morphotypes, fibrils are thought to be particularly extensive in the marine environment, with a large proportion coming from human clothing, ropes, and fishing gear (Andrady, 2011; Cesa *et al.*, 2017).

Clothing remains a significant source of microplastic pollution, as many items are made from synthetic materials such as nylon and polyester. Due to their miniscule size, most washing machines and dryers do not have inbuilt filters that can capture microfibrils, so these escape into the wastewater drains and eventually are discharged into the sea. Browne *et al.* (2011) reported that one single polyester fibre shirt can release 1900 fibrils during a single wash, so this value adds up quickly. As evidenced by the microfibrils found within *M.*

*galloprovincialis* tissues during this study, along with ingested microfibrils reported in other species including – but not limited to – other invertebrates such as *Carcinus maenas* crabs (Watts *et al.*, 2015), fish larvae (Steer *et al.*, 2017), and shorebirds (Lourenço *et al.*, 2017), it is clear that this morphotype is bioavailable to marine organisms, consequently entering the marine food web. While scientific research and the public's understanding of microplastic pollution has grown extensively in recent years, most of this awareness has focused upon other morphotypes such as beads and fragments from larger debris, as evidenced by popular campaigns from environmental organisations including Greenpeace (Greenpeace New Zealand, 2020) and Plastic Oceans (Plastic Oceans International, 2020). Consequently, there now appears to be a gap between what microplastic researchers are finding within the environment and what the public is cognisant of, with regards to the sources of plastic and mitigation solutions.

### *Limitations of Research*

Due to the breadth of information collated within this study, the main limitations of this thesis arose from both time and resource logistical constraints. For the two data chapters (Chapters 2 & 3), time and resource limitations resulted in the sample analyses being conducted through visual microscopic analysis, but without the opportunity for confirmatory spectroscopy. Typically, many studies identify microplastics first through microscopic analysis, then confirmation of synthetic particles with Raman or Fourier transform infrared (FTIR) spectroscopy, which can further characterise the specific polymers of the plastic particles, beyond the simple morphotype. Furthermore, laboratory analysis utilised adapted methods

from previous papers, including Dehaut *et al.* (2016), Zhao *et al.* (2016), and Miller *et al.* (2017). Methodologies such as which separation technique to use, the most effective chemical for degradation of a mussel's soft tissue, best filtration size for microplastic separation, and the type of net most suited to carrying out surface water plankton tows all differed drastically throughout the current available literature, thus making comparisons between my thesis and other examples within literature difficult.

The time and resource constraints also meant that the ability to undertake replication of mussel, beach sediment, and surface water surveys within Wellington Harbour was limited, with the plankton tows only able to give a baseline indication of the abundance of microplastics contained within surface waters once every three months. Mussel and beach sampling was more robust, with multiple samples taken from each site during each collection, however the time it took to thoroughly process each sample meant I was unable to examine a larger sample size within this study. Larger sample sizes would provide a clearer and broader perspective of the overall microplastic pollution load within this coastal environment.

Environmental drivers are a significant factor when it comes to microplastic abundance and distribution, variables such as wind strength, vertical mixing, and seasonal changes in ocean currents (Eriksen *et al.*, 2014) are expected to result in a significant change in marine microplastic load throughout the seasons. Data from chapter 3 sees a peak in microplastic abundance towards the beginning of the year, in the summer and early autumnal months. Investigations into the environmental variables at play within Wellington Harbour would be expected to provide inference into the reasons behind any possible temporal change. Chapter 2 illustrated the temporal change that often occurs in surface water microplastic loads from year to year, which was an aspect unable to be examined in Chapter 3, as a long-term study was not feasible in the scope of this study. Temporal and spatial microplastic research appears to be a recent development in the field, with a handful of studies examining a range of different scales, whether that be investigating the fine-scale daily changes within a small area (Imhof *et al.*, 2017), as well as broader seasonal changes across a coastline (Baechler *et al.*, 2020) and monthly variation in beach sediments across the span of a year, on three different islands (Herrera *et al.*, 2018). The consideration of changes throughout time and space are considered necessary for the development of an overall understanding of microplastic occurrence, as well as future predictions.

Studies investigating annual changes in marine plastic pollution are rare to come by, as they require a significant logistical commitment. Two previous research papers which have carried out such a study are Isobe *et al.* (2019) and Ostle *et al.* (2019). The study by Ostle *et al.* (2019) examined the occurrence of plastics throughout the North Atlantic, over a 60-year period (1957 - 2016). This study also utilised a CPR for its sub-surface water sampling, and reported a clear temporal increase in both microplastics and macroplastics. On the other hand, Isobe *et al.* (2019) used data from Neuston net transects collected between Japan and Antarctica in 2016 to infer previous and future estimates of microplastic abundance and distribution using a numerical model. Both studies are extremely different in their approaches, however the reported results provide strong support for a long-term increase in marine plastic pollution, along with the potential for even greater microplastic loads to be seen throughout the world's oceans in the coming years.

Contamination from outside sources poses a significant risk to microplastic studies, particularly as fibres and fragments circulate in the air and are easily removed from clothing. Many studies cite contamination as a means for error in results, including Li *et al.* (2019), stating that contamination control is an integral detail that must be taken into account when designing an experiment or conducting a field survey. Recent studies have reported that microplastics can travel through the atmosphere, including both urban (Wright *et al.*, 2020) and remote (Allen *et al.*, 2019) locations. Estimations using air mass trajectory suggest that microplastics of multiple morphotypes can cover a distance of over 95 km before they settle in the terrestrial or marine environment, thus illustrating their potential to be a major source of transport for plastic pollution (Allen *et al.*, 2019). Contamination risk throughout this thesis is low, as preventative measures were made by using a limited amount of plastic laboratory equipment, not wearing synthetic clothing during collection or analysis, and covering samples to prevent contamination from the air. In saying that, the samples for Chapter 2 were collected by a third party, the crew of San Aotea II, before being initially processed by NIWA. However, steps to prevent contamination within these samples were taken, including comprehensive training of a dedicated crew member to the deployment and retrieval of the CPR, as well as training in how to appropriately label and preserve the silks (Robinson *et al.*, 2014). Regardless, a small level of contamination is likely to have resulted in a minimal amount of measurement error.



### *Conclusions and Suggestions for Further Research*

This thesis has contributed to the global pool of information on temporal and spatial trends of microplastic loads within the marine environment. With consideration to the Southern Hemisphere, little research currently exists to confirm whether the abundance and distribution of microplastics are comparable to what has been reported in the Northern Hemisphere. The differing responses to temporal and spatial sampling, and the highly dynamic nature of microplastic load in Wellington Harbour and the Southern Ocean demonstrates how integral it is to consider time and space when focussing management efforts. These results also support the consensus that no matter where studies are carried out throughout the globe, marine biota is still at risk of ingesting these foreign particles. Ideally, additional research would establish multi-year studies that further demonstrate the spatial and temporal changes that occur in various regions around New Zealand, along with a continuation of surface water collections between New Zealand and the Ross Sea with microplastics abundance confirmed through the use of FTIR or Raman spectroscopy. Ideally, such research would combine field studies with laboratory experiments, to determine the vulnerability of species in New Zealand and Antarctica to the deleterious effects of synthetic particles. These kinds of investigations would account for some of the areas that could not be examined in detail within this study. Additionally, this research would add valuable information that contributes to the development of management and mitigation advice in order to eventually reduce the negative effects that plastic pollution wreaks upon the local, national, and global marine environment.

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## Appendix

Table A2.1: Collective number of tows undertaken, samples collected, and distance towed (nmi) throughout each annual CPR survey from the FV *San Aotea II*.

Year	Tows	# samples	Distance towed (nmi)
2008/09	7	461	2310
2009/10	6	468	2336
2010/11	8	541	2703
2011/12	5	404	2017
2012/13	8	504	2513
2013/14	9	449	2244
2014/15	8	454	2270
2015/16	7	465	2325
2016/17	6	438	2136
2017/18	7	487	2432
	71	4,671	23,286